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In Vivo Structure of Serum Albumin in its Normal Cat[illegible]

by
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In Vivo Structure and Stability of Serum Albumin in Relation to its Normal Catabolism

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Denne afhandling er i forbindelse med omstående tidligere publicerede afhandlinger af det lægevidenskabelige fakultet ved Københavns Universitet antaget til offentligt at forsvares for den medicinske doktorgrad

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The present thesis is based on the following papers:

I Wallevik K & H T Mouridsen: The turnover in humans of polymerized human serum albumin and of fractions of human serum albumin with different N-F transformation patterns Prot Biol Fluids 1969 16 755-761

II Hvidt Aa & K Wallevik: Conformational changes in human serum albumin as revealed by hydrogen-deuterium exchange studies J Biol Chem 1972 247 1530-1535

III Wallevik K & H T Mouridsen: Detection of metabolic denaturation of serum albumin by gel electrophoresis *Scand J Clin Lab Invest* 1973 31 225-230

IV Wallevik K: Reversible denaturation of human serum albumin by pH temperature and guanidine hydrochloride followed by optical rotation J Biol Chem 1973 248 2650-2655

V Wallevik K: Spontaneous denaturation as a possible initial step in the breakdown of serum albumin in vivo Clin Sci Mol Med 1973 45 665-675

VI Wallevik K: Isoelectric focusing of bovine serum albumin Influence of binding of carrier ampholytes Biochim Biophys Acta 1973 322 75-87

VII Wallevik K: SS-interchanged and oxidized isomers of bovine serum albumin separated by isoelectric focusing Biochim Biophys Acta 1976 420 42-56

VIII Wallevik K: Spontaneous in vivo isomerisation of bovine serum albumin as a determinant of its normal catabolism J Clin Invest 1976 57 398-407

These publications are referred to in the text by their Roman numerals

CONTENTS

Introduction

The motivation for the work which formed the basis for the present survey was the hope of clarifying some of the mechanisms behind the normal in vivo catabolism of serum albumin

The turnover of albumin has been and still is one of the unsolved puzzles of physiology In broad outline one knows 1) that in man about 10 g of albumin is broken down per day (112) 2) that the turnover in an organism in steady state+) proceeds as a reaction of first order with respect to albumin would have done (129) i e the number of albumin molecules degraded per unit time is proportional to the albumin concentration and 3) that the first step in the degradation takes place either within the vascular bed or in a compartment in rapid exchange with it (10 89)

Despite considerable efforts by many groups of scientists it has not been possible to localise the breakdown of albumin to a single organ The prevailing opinion is that albumin is catabolised in all parts of the body by endocytosis mainly by the endothelial cells of the capillaries followed by proteolytic digestion in the lysosomes (89 47 28 108) Quantitative experiments to support this theory however are still lacking

The hypothesis for the normal catabolism of albumin which is advanced in this paper was inspired by the work of the late Linderstrøm-Lang - specifically by his picture of a solubilised protein as a molecule in continuous more or less profound structural fluctuation (87) Thanks to an increasing number of

+) in medical literature often named as metabolic equilibrium or metabolic balance

experiments which support this idea (55 79 118) Linderstrøm--Lang s protein model has gradually won common acceptance It has been suggested that the structural fluctuations play a fundamental role in the function of protein molecules but they may also imply that the complicated molecules pass through structural conformations which are less stable with regard to denaturation (87) Another important contribution in this field has been Linderstrøm--Lang s thesis regarding proteolysis which he had already formulated in 1938 (86) and which is here presented in a slightly modified version: Proteins in their native state are only slowly degradable by proteolysis often even completely resistant to proteolytic enzymes but become susceptible to degradation as a result of partial unfolding; the more unfolded the protein structure the faster the proteolysis These physical-chemical characteristics for globular proteins form the basis for the following working hypothesis for the normal catabolism of serum albumin (I)+): In its native state the albumin is not removed from the extra cellular fluid but during function and the associated changes in the three-dimensional structure the protein becomes susceptible to proteolytic degradation either directly in the blood or after endocytosis

Experimentally the hypothesis was supported by a well known observation from experiments on the normal catabolism of serum proteins: when an injected tracer protein is denatured it is broken down more rapidly than its native counterpart (39 II III) The enhancement of the rate of degradation of denatured albumin is roughly parallel to the degree of denaturation measured by

+) The Roman numerals refer to the author s own work which enters into this paper

physical-chemical methods (39 V) As recently as 10 years ago when the techniques were not so refined as today the measurement of the physiological degradation of a serum protein was usually the only possibility for determining whether it was native or slightly denatured (111 III)

The present paper which is a survey of my own and others' work on albumin falls naturally into two parts:

1) an attempt on the basis of investigations made in vitro to sketch the structure and stability of serum albumin under physiological conditions (II IV V VI VII)
2) an attempt to clarify whether part of the normal breakdown of albumin in the body could be a consequence of in vivo changes in the structure of the protein (I V VIII)

The present exposition will thus be an effort to combine knowledge from two so different fields as the physical chemistry of proteins and medical physiology

The concepts within the two disciplines do not traditionally have the same value However they are most precisely defined in physical chemistry and those definitions therefore are used in this article This has made it necessary to replace some commonly used medical terms with new ones; for example the metabolic stability of a protein has been termed its sturdiness and the term metabolic denaturation has been replaced by biolability

Unfortunately denaturation a concept widely used in the present survey is not clearly defined I will hold to a relatively new definition proposed by Tanford (132): Denaturation is a major change from the original native structure without alteration of the amino acid sequence i e without severance of any of the primary chemical bonds which join one amino acid to another

The definition implies two obvious weaknesses firstly it calls for a definition of the original native structure ; secondly it includes the imprecise formulation a major change which means that the scope of the definition remains a matter of personal taste In spite of these weaknesses however the definition is the most clear-cut and concise one available A more precise definition of the 'native structure of albumin is part of this thesis and will be set forth later

This survey is restricted to a description of those properties of serum albumin which the author has found relevant for an estimation of its structure stability and behaviour in vivo With regard to other physical-chemical properties of albumin (119 107 108) or more specific problems concerned with the measurement of its catabolism (89 119 107 114 115 113) the reader is referred to the survey articles mentioned

The serum albumin of different mammals has such differences in amino acid sequence (124 107) that it can not be taken for granted that it will behave similarly either in vivo or in vitro However human and bovine albumin which have by far been the most thoroughly investigated do not differ substantially from a physical-chemical point of view Thus a distinction will only be made between albumins when physical-chemical differences have been demonstrated or when data are only available for one species of albumin

The molecular structure and stability of serum albumin

An albumin molecule is one single peptide chain of about 580 amino acids the exact number depending on the species The three-dimensional structure is stabilised by 17 disulfide bridges (124) and there is one free SH-group per molecule albumin (53)

The amino acid sequence is almost entirely determined for both bovine and human serum albumin (21 7) The cysteinyl residues are distributed in the amino acid chain in such a manner that the 17 SS-bridges probably form relatively short double loops each including 6 to 43 amino acids (20) The albumin molecule is naturally divided into nine such double loops linked together by a short chain of amino acids (21) The folding of the nine double loops into the three dimensional structure of albumin should thus solely depend on non covalent chemical bonds

The folding of the albumin molecule as if it consists of genuine subunits agrees well with physical-chemical measurements and has actually been predicted solely on the background of such data (IV) Based on observable differences in structural behaviour of the molecule during various considerations the number of subunits has been reduced to 3 or 4 (12 38) which in current terminology are designated as domains (22)

In vitro human serum albumin adjusts to its tightest structure (IV) and the molecule obtains its maximum stability (II) at pH 6-7 and temperatures below 5°C The chemical bonds which keep the above-mentioned domains into their sterical position can be disrupted reversibly by lowering the pH-value to 3 4-3 6 (5) By this NF-transformation+) (5) the molecule loses about 10 percent of the native three dimensional structure as measured from its content of α-helix++) (IV 122)

+) N stands for normal F for fast migration rate in free electrophoresis

++) It must be stressed that the α-helix-content of a protein measured as its capacity to rotate polarised light (optical (Footnote continues at p 12)

The albumin molecule likewise unfolds to a more open and by optical rotation well-defined structure by elevating the pH value from neutral to about 9 (84 49 IV) This loss in structure is also reversible and is called the N-B transition+++) (IV) or the neutral transition (49) The transition from N to B is accompanied by a reduction of the measured α-helix content of albumin of as little as three percent (IV) As it is probably the side chains of albumin which are involved in the transitions mentioned (84) the overall structural change may be much greater than expressed by the loss in α-helix At 37 5°C the N-B transition has a midpoint at pH 7 8 (IV) The position of the midpoint and the steepness of the transition is strongly dependent on Ca^{2+} ions which in concentrations 10 times as high as the physiological displace the midpoint about 0 6 pH units downwards without influencing the N and B state (49)

At 37°C the albumin is in a temperature-dependent transition between molecular states which are not experimentally well-defined (IV) Probably the molecule has its tightest structure at a temperature below 0°C and starts to denature irreversibly above 50°C before the entire three dimensional structure - determined

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(Footnote continued)

++) rotation) only gives a fragmentary picture of the complex structure of the molecule However the method is relatively simple and gives one of the few structural parameters we can measure on solubilised proteins The α-helix content of a protein can not directly be correlated to the stability of the molecule Mutants of a protein e g where only one amino acid in the peptide chain is substituted may well have identical optical rotation but still be significantly different with regard to stability against denaturation (142) Sickle cell anemia is a classic example (103 56)

+++) B stands for basic (alkaline) configuration

by weak non-covalent chemical bonds - is lost At 37°C and pH 7 5 the helix content is 20 percent less than at 5°C (IV)

The pH- and temperature-dependent reversible denaturations take place independent of each other which indicates that different parts (loops or domains) of the albumin molecule are involved in the unfoldings (IV)

Thus at 37 5°C and pH 7 3 serum albumin is involved in relatively steep transitions between different conformations Consequently small variations in either temperature or pH-value will result in appreciable displacements in the equilibrium constants for the reversible denaturations in question (IV)

The net energy which keeps a protein in its ordered three dimensional structure is surprisingly small at least according to the few cases in which it has been experimentally determined (133); thus the free energy (ΔG^{O}) change accompanying the unfolding of ribonuclease and lysozyme from their native structure to a restricted random coil (without breaking any SS-bridge) amounts to only 44 and 60 kJ mol^{-1} respectively (133) As mentioned the unfolding of albumin takes place in several more or less independent steps and for this reason it has not been possible to determine the free energy of complete denaturation of albumin (IV) By hydrogen exchange however it has been possible to get an impression of the amount of the energies which hold the molecule together (II) At pH 6 5 the albumin has its greatest stability but less than 25 kJ mol^{-1} (ΔG -value) is still sufficient to open the molecular structure enough to let the surrounding water enter and come into contact with 60 percent of the peptide bonds of the molecule (II) Increasing the pH-value to 7 6 makes the albumin become more unstable in as much as 70 percent of the peptide bonds are now regularly in contact with the water These

measurements are carried out at 25°C; increasing the temperature to 35°C causes a further five percent of the molecule s interior to be exposed to the surrounding solution (II) The magnitude of the 25 kJ mol^{-1} can be evaluated in relation to the fact that it takes 13-29 kJ mol^{-1} to break a single hydrogen bond and that the weakest type of covalent bond (the disulphide bond) requires about 200 kJ mol^{-1} (139)

There are appreciable differences in the energies which stabilise the different parts (domains) of the albumin molecules (II IV) At pH 6 5 and 25°C about 40 percent of the peptide bonds are as mentioned protected against the solvent by ΔG values higher than 25 kJ mol^{-1} while 35 percent are protected by energies below 17 kJ mol^{-1} Among the remaining 25 percent peptide bonds there is an equal distribution of ΔG values from 17 to 25 kJ mol^{-1}(II)

As mentioned the albumin molecule unfolds from the N to the B state by increasing the pH of the solution from neutral to around nine When albumin is in the B-state it more easily denatures irreversibly than in the N state Also the heat-induced loosening of the albumin structure renders the molecule less sturdy against irreversible transformation

These conclusions are based on the following observations The rate at which albumin is cleaved by trypsin increases much more by a stepwise change of the temperature from 5 to 40°C and of the pH value from 7 to 8 than can be accounted for only by the increase in activity of the enzyme accompanying the mentioned parameters (137) Furthermore the specific denaturation process which is called intramolecular SS-interchange (which will be discussed in detail later) runs slowly at pH 7 but the reaction

speeds up by increasing the pH value of the solution and reaches a plateau at pH 8 5 (130) The rate of irreversible heat denaturation and denaturation by storage change likewise from a minimal to a maximal level in the same pH interval (138 V)

Beyond this the N-B transition is important for the specific as well as the unspecific binding characteristics of albumin As an example there is by transition from N to B in the pH interval from 7 to 8 an increase in the binding constants corresponding to an enhanced affinity for the amino acid tryptophan (91) and for Ca^{2+} ions (31)

As a last phenomenon I shall mention that binding of different compounds naturally present in the body may influence the albumin structure in a stabilising manner (see below) analogous to the effect on enzymes frequently seen after binding of substrates

Thus for the present there is nothing which indicates that albumin in vitro close to physiological conditions should have a structure, stability and mobility which differs substantially from what can be obtained in vivo Consequently serum albumin must in vivo be in equilibrium between folded and partially unfolded molecules

The heterogeneity of serum albumin The appearance in vivo and the catabolism of isolated subfractions

Industrially produced serum albumin of highest purity appears immunologically homogeneous; nevertheless by physical-chemical methods it can be fractionated into sub-populations and is thus heterogeneous (121 37 VII) Since the invention of the ultracentrifuge it has been known that albumin is heterogeneous and the continuous refinement of physical-chemical methods have

resulted in the characterisation of still new classes of heterogeneity Only in the last decades especially by introduction of refined chromatographic techniques (105 62) and of isoelectric focusing (131 VI VII) has it been relevant to try to discover whether the heterogeneity of albumin occurs in vivo as well (I VIII)

The most important observations and formulations of problems with respect to the heterogeneity of albumin resulted from the work of Joseph Foster and his collaborators at Purdue University Lafayette Indiana (5 6 37 84 99 121 122 123 130 143) but other groups have also contributed notable discoveries King and Spencer have explored the heterogeneity connected with the free SH-group of albumin (71 72 125) and Fuller-Noel and co-workers were the first to draw attention to the heterogeneity originating from oxidised forms of albumin (41 62)

In the present survey I shall primarily deal with the subfractions of albumin which are fairly well characterised and shall especially try to estimate whether corresponding fractions are present in vivo A survey article on the heterogeneity of albumin has recently been published (61)

Dimer and polymer albumin

All preparations of albumin contain 5-15 percent dimer albumin and often a few percent trimer and higher polymers (104) Less than 50 percent of the dimer is formed by intermolecular disulphide bonding (62) The remaining part is dimerised by non-covalent binding forces as it dissociates in a denaturing medium such as one percent sodiumdodecylsulphate under non-reducing conditions

Purified dimer and trimer human albumin have a turnover rate in man which is respectively 20 and 40 percent higher than for

monomer albumin (I) These rate differences are so small that one should expect to find dimer and trimer albumin in the blood if the polymer species were produced in vivo However it has not been possible to trace polymer albumin in fresh bovine and human plasma (3 I) On the contrary it is found that purified radioactively labelled dimer albumin is partially converted to monomer in vivo and that the monomerized albumin has the same turnover in man as the native albumin (I)

Substances bound to albumin

Serum albumin has the capacity to bind to a large number of naturally occurring substances and drugs Many of these compounds - e g fatty acids (46 26) bilirubin (57) tryptophan (91) and copper (75) - are bound with large affinity to a well-defined section of the albumin molecule i e distinct binding specificity is exhibited Marked advances have been made during recent years with regard to the identification of binding sites on the albumin molecule for different compounds (126 108)

For each of the different substances the binding to albumin results in physical-chemical changes which originate partly from the properties of the bound substances per se (126 VI) and partly from conformational changes induced in the albumin molecule (127 109 27 11) These relatively small molecular fluctuations can depending on the properties of the bound substances be either a loosening of the structure (pharmaca and other unphysiological compounds)(27 11) or a tightening (127 11) The stabilising effect of fatty acids (15) tryptophan (88) and divalent metals (48) can also be reflected in a general protection of albumin against irreversible heat denaturation (15 88) proteolytic degradation (74 76 48) and intramolecular SS-interchange (123

99) When fatty acids are bound to albumin the molecule is to a certain extent protected against unfolding from the N to the F state (122) A similar effect of fatty acids has not been observed on the N-B transition (143)

One of the physiological functions of albumin is to keep free fatty acids in a solubilised form in the plasma (46 16) All industrially prepared albumin preparations contain at least 1-2 molecules of fatty acids per molecule albumin (26) When esterified ^{14}C-labelled fatty acids are bound to albumin _in vitro_ and the complex injected intravenously the ^{14}C-activity in plasma is reduced to one percent of the initial value in 10 min At this time the majority of the radioactivity can be recovered from the adipose tissue (16) This indicates that fatty acids bound to albumin are rapidly exchanged between blood and tissue

The alterations in the physical-chemical stability of albumin which result from binding and release of different compounds must also manifest themselves _in vivo_ As a consequence of the rapid exchange in blood of substances with binding affinity to albumin it is difficult to interpret catabolic experiments with albumin made homogeneous with respect to a bound compound However it has been shown that defatted rabbit and bovine albumin are catabolised at the same rate as the nondefatted protein (VIII) As mentioned the pH-value at which albumin unfolds in the N-F transformation is dependent on the number of fatty acids bound to the individual molecules (122) Fractions of human albumin which have different N-F transformation characteristics are not found to differ metabolically (I)

The free SH-group of albumin

In industrially prepared albumin the number of free SH-groups

per molecule protein vary from 0 3 to 0 6 and is never larger than 0 8 even with the most meticulous purification methods (71 3 35) After a mild reduction with SS-reducing substances such as 0 1 M thioglycolate or mercaptethanol the value of 1 mol SH per mol albumin is measured (71 3) The unpaired SH-group is localised to amino acid No 34 in the peptide chain counted from the amino terminal end (72) The free SH-group of albumin reacts rapidly with low molecular weight SH-compounds at pH values larger than pH 8 under formation of mixed disulphides whereas the same reactions proceed slowly at pH-values below 7 (65 32) At physiological pH and temperature in vitro the SH-group of albumin is blocked after 20 hours with a 5 molar surplus of cystein in the solution (32) This is in contrast to the reaction amongst low molecular weight SH-compounds which react quickly at pH-values as low as 6 to 7 (65) These observations led to the supposition that the free SH-group at pH-values below 7 is sterically protected and is exposed to the solvent through the N-B transition (41) Spin labelling indicates that the SH-group in albumin is situated in a 0 95 nm deep pocket (54)

The explanation for the reduced molar ratio of the free SH--group in albumin preparations is either the formation of mixed disulphides by reaction with low molecular weight thiols in serum (71) or oxidation of the SH-group with molecular oxygen to sulphenic acid (41 VII)(see below) The molar ratio decreases during storage which depends further on the storage conditions of the serum from which the albumin is isolated (3) On the background of these findings it has been unclear whether the unpaired SH-group was already partially masked in vivo or whether this happened only after the blood was taken (35) Iodoacetamide reacts quickly with

the free SH-group of albumin already at pH 6 5 (62) By taking blood directly into phosphate buffers 0 1 M pH 6 5 containing different concentrations of ^{14}C-iodoacetamide with known specific radioactivity it is determined that the amount of iodoacetamide bound to albumin after it has been isolated from the serum is consistent with a binding ratio of 0 6–0 7 mol iodoacetamide per mol albumin (Wallevik unpublished results) Thus it must be regarded as most probable that the free SH-group of albumin is also partially blocked _in vivo_

When the free SH-group of rabbit serum albumin is blocked with ^{14}C-cystein and this albumin preparation is injected intravenously into a rabbit the cystein will be cleaved from the albumin within 15 min This means that the free SH-group _in vivo_ is in constant exchange with low molecular weight SH-compounds present in the blood probably in the course of their transportation It has also been shown that peptides with a free SH-group _in vivo_ can be linked to albumin by means of an intermolecular SS-interchange reaction (81)

Intramolecular disulphide interchange in albumin

The seventeen SS-bridges of albumin can by so-called disulphide interchange reactions (SS-interchange) change position within the molecule (3 123) This process is catalysed either by the albumin molecules own free SH-group (99) or by low molecular weight thiols in the surrounding solution (VII) Bovine serum albumin which has been submitted to such an intramolecular SS--interchange differs from native albumin by having an isoionic point 0 1 - 0 2 pH units higher (VII) and by a 5 6 percent expansion of the molecule (130 VII) The formation of SS-interchanged albumin is stated to be a reversible process The reaction rate

measured for the reverse process was considerably slower than the one calculated from the determined equilibrium constant and the rate-constant for the forward process The reason for this contradiction was as explained by the authors (130) that it had not been experimentally possible to start the reverse process with homogeneous preparations of SS-interchanged albumin This explanation obviously makes it questionable whether equilibrium actually was obtained between native and SS-interchanged albumin (130)

Purified ^{125}I labelled SS-interchanged bovine serum albumin is catabolised faster <u>in vivo</u> than is native albumin Simultaneously it is reversed to albumin with the native isoionic point (VIII) The albumin which is reversed from the SS-interchanged form has the same physiological disappearance rate as native albumin (VIII)

Intramolecular SS-interchanged albumin is metabolically heterogeneous and the distribution of disappearance rates in purified preparations depend on the circumstances under which the SS-interchanged albumin is produced (VIII) Thus the SS-interchanged albumin which is isolated from industrially manufactured bovine albumin consists of two metabolically distinct fractions The main part has a half-life of about three days but a not negligible fraction has a half-life of 18 days which is close to the value for native albumin despite the fact that this fraction maintains the isoionic points of the SS-interchanged isomers (VIII) If on the other hand the SS-interchanged albumin is formed <u>in vitro</u> by catalysis with cystein the metabolic heterogeneity is enhanced and the purified isomers contain a surplus of at least 25 percent albumin which is catabolised with a half-life of less than half a day (VIII)

Ten percent of the albumin in freshly taken bovine serum has isoionic points identical to the SS-interchanged isomers A corresponding amount of ^{125}I albumin purified with respect to the native isoionic point will after injection adapt to isoionic points identical to SS-interchanged albumin (VIII) As the in vivo conditions represent a milieu which in vitro promote the SS-interchange reaction (66) it must be considered as probable that albumin in vivo is also converted reversibly to the metabolically more labile SS-interchanged species and that these processes result in a mixture of the two components in the concentration ratio ten to one (VIII)

Oxidized albumin

Native bovine serum albumin incubated in vitro in the pre sence of atmospheric oxygen at conditions otherwise close to physiological is modified irreversibly to albumin with an isoionic point lower than native albumin (VII) During the process the free SH-groups of albumin are oxidized but no dimer albumin is formed (41 VII) However it can not be the oxidation of the SH-group which causes the displacement of the isoionic points as the modified cysteinyl residue can be reduced back to free SH by a mild reduction without simultaneous restoration of the native isoionic point (VII)

The oxidative modification of bovine serum albumin is localised to the primary structure (VII) Until now however it has not been elucidated which amino acids are involved in the reaction It might be desmidated amino acids (125) or oxidation of broken SS-bridges to sulphonic acid ($R-SO_3H$)(134 41) Albumin modified by molecular oxygen catabolises 10-30 percent faster than native albumin and does not regenerate in vivo albumin with native

isoionic point (Vallevik unpublished) It has not been possible to decide whether irreversibly oxidized bovine albumin is present in vivo since the isoelectric focusing by which it is demonstrated itself produces the oxidized form (VII) From isoelectric focusing of normal serum it might however be concluded (VIII) that oxidized bovine albumin with isoionic points lower than native albumin can not be present in vivo in concentrations higher than a few percent (Vallevik unpublished)

Denaturation of albumin during storage

By storage of rabbit serum albumin in vivo as close to physiological conditions as possible it has been demonstrated that albumin denatures The amount of denatured molecules and their degree of denaturation is increased by the incubation time (V 110) The reactions involved in this denaturation have not been clarified but probably both SS-interchange and oxidation are involved After publication of the referenced paper (V) proteolytic activity has been demonstrated in several industrially produced albumins (6) Sodiumdodecylsulphate polyacrylamide gel electrophoresis repeated on the rabbit albumin samples which constituted the material for the observations in reference V and which had been stored at -20°C for 5 years excluded proteolysis as the possible cause for the demonstrated denaturation (Vallevik unpublished)

Additional albumin heterogeneities with possible relevance for the in vivo catabolism

Chemical modification of a few amino acids in the peptide chain of albumin may possibly take place in vivo Oxidation of albumin with molecular oxygen is an example of such a possibility

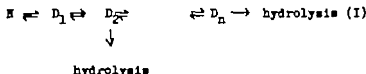
(VII) It has been demonstrated that acetyl salicylic acid in pharmacological doses in vivo acetylates the amino acid lysin in albumin (50) and especially is lysin No 119 counted from the amino end of the peptide chain exposed to this modification (136) This type of reaction may possibly take place in the body between different metabolites and albumin

Finally it is indicated that a fraction of bovine albumin has arginine added to the NH_2-terminal end - a chemical modification which can only be envisaged as taking place in vivo either during protein synthesis or postsynthetically through enzymatical addition (83)

Albumin with physiologically modified amino acids has not yet been purified from plasma and consequently it is difficult to predict any possible influence on the function or catabolism of serum albumin However it can be mentioned that rabbit albumin or human albumin acetylated with one mol acetyl salicylic acid per mol albumin can not be distinguished metabolically during the first week after injection in rabbits (Vadstrup and Wallevik unpublished results)

Experimental observations with regard to the working hypoth sis

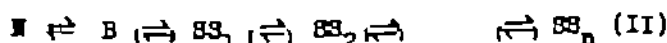
The hypothesis for the physiological degradation of serum albumin was described in the introduction and can be formalised in the following reaction scheme (I V VIII):



Reaction scheme I expresses that the native conformation+) *in vivo* can be converted reversibly into different denatured forms D_1 D_2 D_n of albumin According to the theory N should only be catabolised after preceding transformation to one of the denatured forms

In the previous section it was shown as probable that serum albumin *in vivo* does not maintain its tightest structure but is involved in various reversible processes which imply transitions to more open and less sturdy conformations These openings of the structure are provoked by changes in temperature and hydrogen ion concentration and by presence of various ligands It was further shown that albumin *in vivo* is converted reversibly into different probably intramolecular SS-interchanged structures The SS-interchanged albumin probably has the B-form of the mentioned N-B transformation as a basis (99 130)

This information can be formalised in the reaction scheme:



$$N \rightleftarrows B \ (\rightleftarrows)\ SS_1 \ (\rightleftarrows)\ SS_2 \ (\rightleftarrows) \qquad (\rightleftarrows)\ SS_n \quad (II)$$

where B is the basic configuration and SS_1 SS_2 and SS_n represent different degrees of SS-interchange The brackets around the reverse processes imply that both *in vitro* and *in vivo* it has been demonstrated that not all SS-interchanged albumin is reversed to native protein (130 VII)

+) The recognition that albumin is also heterogeneous *in vivo* makes a re-evaluation of the concept native albumin necessary In the following discussion I will keep to the physical-chemical definition when talking about physiological conditions and consider native albumin as the configuration of the molecule which under the *in vivo* conditions is most resistant to denaturation (132)

Reaction scheme II contains only the experimental findings with respect to the SS-interchange in vitro and must be greatly simplified compared to the in vivo situation In vivo for example the temperature- and the ligand-induced conformational changes must enter into the chain of processes in an unknown order

The rate constants of the isolated processes in reaction scheme II must be very different from each other as the processes in the N-B transition are fast reacting (t/2 < 1 sec)(116) while the SS-interchange reactions are slow processes (hours to days) (130 VII)

The fundamental agreement between reaction scheme I and II is evident

With regard to the second part of the hypothesis - that the denatured forms of albumin in reaction schemes I and II should be less resistant than native albumin to in vivo degradation we have the following experimental basis:

1) as mentioned it has been shown in vitro that the B-form and the temperature unfolded subspecies are more susceptible to proteolytic degradation than native albumin (137)

2) The most important observation in this connection however is that SS-interchanged albumin in vivo is catabolised faster than native albumin (VIII)

From the parameters determined by the turnover of radioactive labelled SS-interchanged albumin in cows the amount of native albumin which might be catabolised through a possible preceding SS-interchange reaction might be quantitively estimated (VIII)

Ten percent of the albumin has in vivo isoionic points corresponding to SS-interchanged albumin If this amount is catabolised with the same half-life of three days as determined for a similar

fraction isolated from industrially manufactured albumin about half of the normally catabolised albumin will have to pass through an SS-interchange reaction beforehand (VIII)

As mentioned above a considerable amount of the SS-interchanged albumin isomer formed by catalyses with cystein is catabolised with a half-life of less than half a day If only two percent of the albumin in the organism catabolises this fast the degradation of this fraction would account for about 50 percent of the total albumin turnover (VIII)

The cysteine catalysed albumin isomer has not as such been demonstrated in the blood Nevertheless the estimation just mentioned is included in this paper to draw attention to how small the amounts of fast degraded albumin are which need be present in the blood as part of a reaction chain of type I, to account for appreciable fractions of the total albumin turnover

It will be difficult to directly demonstrate such small amounts of a modified albumin in the blood However if an isomer after parenteral administration can regenerate native albumin this will be a fair indication that the isomer might be propagated in vivo When the renaturation takes place in vivo the denaturation process must be practicable as well

As mentioned previously the particular cysteine-catalysed albumin isomer does actually renature after injection (VIII) This, however together with the fact that the physiological milieu is compatible with the SS-interchange reaction (66) is so far the only indication that the fast degraded SS-interchanged isomers could be physiological products

The final hydrolyses of serum albumin after spontaneous physiological denaturation

The proposed model for the normal degradation of albumin has up to now dealt with the initial steps in reaction scheme I A further requirement for the relevance of the theory is that the denatured albumin molecules ultimately be hydrolyzed by the mechanisms normally applied by the organism to degrade denatured proteins (I V VIII)

According to the thesis of Linderstrøm-Lang there should be no problems in sorting native protein molecules from denatured when only proteolytic enzymes are present (86)

Before a closer discussion of the final possibilities of hydrolysis it should be mentioned that from turnover experiments with labelled denatured albumins (39 III) two degrees of biolability might be distinguished

1) Totally biolabile albumin where the molecule before the injection is transformed to such an extent that it is cleared from the blood in a few minutes

2) Partially biolabile albumin where the rate of degradation of the molecule is moderately enhanced compared to native albumin but the denaturation is so minor that it frequently can not be measured by common physical-chemical methods

Extracellular hydrolysis: Proteolytic activity in the blood is slight because of an excess of protease inhibitors (82) However proteolytic enzymes associated with blood coagulation (e g plasmin) function extracellularly and proteolytic activity can be measured directly in fresh plasma (6) Protease and esterase activity are as previously mentioned also recognized in even

highly purified albumin preparations an activity which also in vivo could possibly be in close contact with albumin (6) If proteolysis occurs in the extracellular space per se however it is experimentally difficult to trace the resultant peptides as they probably will be rapidly eliminated from the circulating blood

Intracellular hydrolysis: The second possibility - which on the other hand has good experimental support - is that the denatured albumin is picked up by means of endocytosis into different cell--systems of the organism and hydrolyzed after fusion of th endocytic vesicles with the proteolytically active cytoplasmic lysosomes (93 47 30 28) As endocytosis of native albumin especially in the endothelial cells is considered to play a crucial role in other relevant theories on normal albumin catabolism as well (108 51 17 18) the problems connected with physiological endocytosis of albumin justify a closer discussion

Endocytosis of serum albumin

The process of endocytosis involves the transport into cells of extracellular media by means og invagination of the surface membrane to form a vesicle which then enters the cell The transport of mat rial by endocytosis consequently has two vehicles: 1) the cell surface with its specific adsorptive capacity (adsorptive endocytosis) 2) the extracellular solution which is passively surrounded by the released piece of cell membrane (non-specific liquid endocytosis) (9 58 1 25)

The classical term phagocytosis indicates uptake of particulate material into cells and is mainly restricted to the reticulo-endothelial system In its original designation pinocytosis implies

uptake of solutes However there is a continuous spectrum in the size of the ingested material from small molecules through macromolecules aggregates to bacteria hence the common term endocytosis is now often used to cover these varied processes (58 25 1) However there are considerable quantitative differences between the two processes as well as differences in specificity among various cell systems (25 1)

When totally biolabile albumin is injected intravenously it is picked up by the Kupffer cells of the liver (8) with a half-life of 6 5 min (102) It has no influence on the uptake whether the totally biolabile albumin is monomer or polymer (23) and the uptake is probably not specific for biolabile albumin but for denatured albumin as a whole (100)

If the albumin in reaction scheme I attains a degree of denaturation matching that of totally biolabile albumin it should also be taken up by endocytosis in the Kupffer cells As only 10 percent of the normal catabolism of albumin takes place in the liver (51) it is not possible to account for the final hydrolysis of albumin by endocytosis in this organ alone

However endocytosis is beginning to be recognised as a common cellular mechanism of transportation in eukaryotes (9 101 52) and it has been demonstrated that albumin in vitro is taken up in a variety of cells: endothelial cells+) adipose cells (29) fibroblasts (128), liver cells (47 93 30) kidney renal tubules (14) wandering phagocytes (13 33 94 73) rat yolk-sac epithelial cells (140 141) and cancer cells (117 92 106)

In most of these investigations the primary interest has been the cellular mechanisms involved in the process of endocytosis

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For technical reasons the albumin used has been labelled with iodine to such a specific radioactivity that it can be stated with certainty that if the labelled albumins had been used in catabolic studies in whole animals they would have turned out to be biolabile+) (39) In the few investigations where the authors were aware of the possible influence of the albumin structure on endocytosis it has been shown that native albumin is not taken up by migrating phagocytes (13 73) or amoebes (24) in contrast to denatured albumin which is rapidly internalized In liver cells (92) and epithelium from the rat yolk-sac (95 96) a positive correlation is furthermore demonstrated between the degree of denaturation of the tracer albumin and the rate of endocytosis This increase in the rate of endocytosis parallel to the degree of denaturation is due to an increased adsorption of the modified albumin to the cell surface and not to an increased formation of endocytic vesicles (95 96 141)

If these observations can be transfered to other cell systems a hypothesis for the endocytosis of extracellular proteins can be advanced which follows the same principles as the thesis of Linderstrøm-Lang for the proteolytic degradation (86): native proteins are not internalized in cells by specific endocytosis; however when denatured they are taken up specifically by endocytosis at a rate which is positively dependent on the degree of denaturation

The molecular mechanisms involved in the selection of the denatured molecules by the c ll-surface-complex can be explained

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+) The biolability is a result both of an xcessiv degree of iodination (i e number of iodine atoms per molecule albumin) and a high dose of irradiation from the bound radioactive iodine

without difficulty on the basis of common knowledge of protein denaturation and protein-protein interaction (133 63) For example in binding of protein substrates to the active centre of proteolytic enzymes the binding affinity is proportional to the length of the peptide chain bound (64) A biolabilisation or a denaturation of albumin will be associated with a partial unfolding of the molecule which means that a larger number of amino-acid side chains are exposed to the solvent and become accessible to binding to the surface of the membrane most likely mainly by hydrophobic interaction (64 63)

If endocytosis is involved in the normal catabolism of albumin it must be the degree of denaturation and not merely an aberrant albumin structure which determines the rate of endocytosis This conclusion can be derived from the experimental finding that human albumin as long as it is native is not catabolised faster in, for example rabbits (39) and dogs (44) than the homologous albumins - although the structure of the human albumin deviates so much from the homologous that the immune system of the animal is activated This sensibilisation of course has the consequence that after a week of normal catabolism of the human albumin it is rapidly eliminated from the animal (39)

In the theories based on endocytosis as an important element in the normal turnover of albumin endocytosis is mainly considered unspecific (108 89 119) The consequence of such an unspecific liquid endocytosis would be that native and partially biolabile albumin are internalised at the same rate In order to explain the small differences in degradation rate which are frequently seen amongst albumin preparations it has thus been necessary to postulate a considerable flow of albumin through the cells in

the process of which the denatured molecules should be more quickly hydrolysed in the lysosomes than the native albumin (108 28) The theory presupposes that the vesicles which come in contact with the lysosomes are again released and exocytosed a sequence of events which has not thus far been demonstrated experimentally (59)

The migration of endocytic vesicles and the transport of denatured protein through the endothelial cells of the capillaries have been demonstrated by electronmicroscopy (120) On the other hand it has been argued convincingly that this mechanism of transfer can not quantitatively play any major role in the exchange of albumin between the intra- and extracellular space (80)

Adsorptive endocytosis where the affinity of the cell-surface -complex for albumin - and consequently the rate of endocytosis - depends on the protein structure seems thus to be the most likely mechanism for sorting denatured albumin from native prior to the final intracellular hydrolysis

The present potential of purifying well-defined modified albumin (VII) together with the possibility of growing endothelial cells <u>in vitro</u> (60) and determining their uptake of tracer proteins+) should make it possible to verify whether the correlation between albumin structure and rate of endocytosis is also valid for endothelial cells and furthermore to decide whether native albumin on the whole is endocytosed by this cell system <u>in vitro</u>

Discussion of the working hypothesis in relation to the established facts on the normal catabolism of serum albumin

No points in the proposed theory on the degradation of

+) Joyner W L and Moriarty G M personal communication

albumin are in conflict with our present knowledge of catabolism of albumin As mentioned in the introduction the degradation of native albumin in a body in steady state proceeds as a reaction of first order with respect to albumin (129) Protein denaturation usually follows first order kinetics (85 78); this is also the case for the SS-interchange reaction in albumin catalysed by its own free SH-group (130)

It has been demonstrated that native albumin accumulates in wound tissue Degradation of the trapped albumin does not take place before it has returned to the normal blood circulation (97) When returned to the circulation the turnover of albumin which has been deposited in a wound is normal (98) These findings support that 1) albumin is only catabolised when participating in the normal exchange between the intra- and extravascular space and 2) a tissue with marked phagocytic activity (macrophages leucocytes) is not especially catabolically active towards native albumin

McFarlane who is one of the pioneers in the exploration of the normal turnover of serum proteins (89) has recently measured the catabolism in rabbits the first 24 hours after injection of radioiodinated albumin and demonstrated that radioactive iodine during the first hours is apparently released from the tracer albumin with only half the rate of that obtained after 24 hours (90) From this data McFarlane and collaborators conclude that an albumin pool somewhere in the animal has to be in balance with the labelled albumin before it is degraded at the maximum rate (90) In the discussion on where in the body this pool may be localised McFarlane assumes that all the albumin is catabolised by a single mechanism The experimental results he obtained however could just as well be explained by assuming that albumin is

degraded by various mechanisms which may degrade albumin at different rates and until the system has reached a steady state may be recognized as displaced in their release of radioactive iodine to the iodine pool of the body Experimental observations do exist which support the suggestion that the catabolism of albumin could take place via different mechanisms (119)

Further possibilities for the catabolism of serum albumin

Albumin may escape from the organism by filtration or secretion both to the internal and external surfaces of the body (for survey see ref 119) However within normal physiological conditions only the loss of albumin into the gastrointestinal tract might play any quantitative role

In 1960 it was estimated that about 50 percent of the turnover of albumin in a dog could be explained by exudation (leaking out) of serum proteins into the gastrointestinal tract (44 45) and since then this mechanism for the degradation of albumin has been under discussion The original investigations have been criticised for the surgical injury considered to give rise to an unphysiological leakage of serum proteins into the intestinal lumen The substance of this criticism was apparently confirmed by later measurements on the turnover of chromium labelled albumin in healthy individuals (70 135) In th se experiments it was found that less than five percent of the normal albumin degradation could take place in the intestinal tract The basis for the experiments was that free chromium is not absorbed from the intestine which means that the amount of chromium in the feces after intravenous injection of chromium-labelled albumin should be a direct measure of the amount of chromium-labelled albumin which

has escaped to the intestine However there are substantial sources of error in the experiments: the labelling with chromium per se denatures the albumin (135 115) and it is not known whether chromium even when it is bound to albumin can be excreted into the intestinal tract in healthy individuals especially when it is known that the free chromium can not be resorbed

Leakage of serum albumin into the gastrointestinal tract as a process of quantitative importance in physiology however has recently been reinvented on the basis of gentle and careful measurements through a gastroscope and of introduction of soft probes orally into the intestines of healthy persons (19) From these experiments it was again calculated that about 50 percent of the breakdown of albumin proceeds by proteolysis of the protein in the gastrointestinal tract (19) If the remaining part is catabolized by for example a spontaneous denaturation and hydrolysis in the vascular bed it may be presupposed that there might be a displacement in time before the tracer iodine released by the degradations of albumin, is balanced with the total body pool of iodine

Other examined sites for the catabolism of albumin are liver and kidney By perfusion experiments on these organs it has been estimated that less than 10 percent of the total albumin degradation has its origin in each of these organs (51 68)

Discussion of th hypothesis in relation to the catabolism of s rum albumin und r pathological conditions

Generally speaking conditions known to increase the degradation rate of albumin in the body (36 114 115 107) and which can not be explained by mechanical loss of albumin will imply an increased probability for protein denaturation as well

At plasma concentrations of albumin which vary 10 g per liter around the normal value it is found that as is characteristic for first order reaction kinetics the amount of albumin catabolised per unit time depends only on the concentration of albumin in the plasma (119 43) Nevertheless at very low plasma concentrations of albumin a smaller and at artificially increased concentrations a larger first order rate constant has been obtained (2) indicating that the degradation of albumin at extreme albumin concentrations is regulated (18 43 67)

As the basis for the discussion of a possibly regulated degradation of albumin I have selected analbuminaemia an extremely rare (12 cases reported in the literature) but reasonably well examined genetical abnormality (40 69 43) The concentration of serum albumin among these individuals varies from 0 2 to 0 4 g per liter in contrast to the normal 40 g per liter (43) The cause is a defect in the synthesis of albumin as the rate of degradation in these persons in their individual steady states is 3 to 7 times slower than in normal individuals When the albumin concentration in these individuals is increased to a normal level by infusions the slow rate of degradation is maintained in some of the cases (40 43) while in oth rs the rate is increased to values characteristic for normal persons (69)

These obs rvations are not directly compatible with albumin degradation as a simple first order reaction which would imply that the relative rate of degradation should be independent of the albumin concentration On this background a theory for normal albumin degradation has been put forward which is in line with the established concentration-dependent degradation of IgG (17 18 67) Briefly the theory holds that degradation and transport of serum protein over various tissue barriers has a common basis (18 67 43)

However in contradiction to the theory based on nonspecific liquid endocytosis (see previous section) specific receptors for the various serum proteins should be available at the cell surface (18 67) The number of these receptors should be limited Proteins bound to the receptors should be transported intact through the cells during the endocytic process while all the proteins which passively accompany (nonspecific liquid endocytosis) should be susceptible to the proteolytic activity of the cell and thus be degraded (18)

At such low plasma concentrations of albumin as are found with analbuminaemia the receptors on the cells should not be saturated and consequently most albumin should be transported intact through the cells while a considerable part of the albumin in normal individuals should be unbound and thus be degraded In the case of IgG such IgG-membrane complexes have been demonstrated in homogenates of the intestines from neonatal rats (67) and they might be present in other cell membranes in adult mice (67) but such complexes are not found for albumin or other serum proteins either in the investigations mentioned or in any other investigations (42)

If the hypothesis of a concentration-dependent degradation of albumin should be brought in agreement with the previously mentioned observations 1) that cells have a special affinity for denatured albumin and 2) that denatured albumin <u>in vivo</u> always is degraded more rapidly than the native protein it will be necessary to postulate additional cellular mechanisms for sorting Either a) only the native albumin is protected by the coupling to the receptors while the denatured albumin is degraded although it is membranebound or b) specific receptors exist for both native and denatured proteins in such a manner that these can be processed differently by the cell

However none of the hypotheses so far advanced for the normal degradation of albumin explain why the turnover in some individuals with analbuminaemia remains slow even after the albumin concentration in serum is artificially brought to the vicinity of the normal (43)

A mechanism for the degradation such as that sketched in reaction scheme I however involves several reversible processes where changes in the concentrations of the single components would be able to regulate the overall process As an example the slow disappearance rate of albumin in albuminaemic persons in their habitual steady state could be explained by the high concentration of free fatty acids in plasma also observed in these individuals (69) The high plasma levels of free fatty acids could displace the concentrations in the reaction: albumin + free fatty acids ⇌ complex (albumin-fatty acids) in favour of the bound form of albumin This form is as mentioned earlier partially protected from denaturation and might consequently also have a slower catabolism

However it must be stressed that analbuminaemia is a disorder which might mobilise rather special mechanisms in the individuals concerned which implies that it is questionable to extrapolate to normal conditions The few cases reported could also have different genetical backgrounds and thus a different etiology

Not only are the experimental facts about the physiological degradation of serum albumin sparse but different investigations of the same problem have - as would have appeared - often given conflicting results The current discussion on the degradation of serum proteins is, as illustrated, encumbered with many unknown factors These again make it necessary to employ many assumptions which might be difficult or impossible to substantiate In this respect

the work presented here and the discussion of its results are no exceptions This presentation has dealt with a fragment which I have examined in relation to the whole Only additional experiments might disclose whether the proposed location of the result within the normal catabolism of albumin is correct

Conclusions with respect to the normal catabolism of serum albumin

The main objective of the preceding exposition has been to point out that denaturation of serum albumin can take place in vivo as a consequence of the structure and stability of the molecule and to emphasise this mechanism as a probable and in case quantitatively important factor in the normal degradation of albumin

The total loss of albumin by the mechanisms described in the literature is estimated to account for around 50 percent of the total catabolism (68) If an additional 50 percent is degraded through the physiological denaturation rendered probable in the present paper the main part and possibly all of the normal albumin degradation should be accounted for Together with the probable loss of albumin into the gastrointestinal tract the in vivo denaturation described completes the picture of the albumin catabolism as a result of an inevitable unspecific randomised loss of albumin If that is the case the only factor common to these two mechanisms is that albumin ultimately is hydrolysed by the proteolytic enzymes of the body in such a way that the amino acids are recycled

General remarks concerning physiological postsynthetic modification of proteins

The primary structure of a protein determines its threedimensional folding and thus its function (4) The consequence of the observations presented could be that the primary structure by

deciding the stability of a protein at the same time determines its lifespan in the body

The comparisons made between the stability of albumin _in vitro_ and _in vivo_ have often been criticised on the basis of the assumption that any incubation _in vitro_ under conditions close to the physiological involves a heavier strain on the protein than is so in the physiological state An essential result of the investigations reviewed is the indication that even a molecule as sturdy as serum albumin has neither its most compact structure nor its optimal stability under _in vivo_ conditions Therefore returning once more to Linderstrøm-Lang (87) we generally have to reconsider the commonly accepted but never proven concept of physiological conditions as especially stabilising for proteins It is probably nearer the truth that a functioning protein is a population of molecules with small differences in structure and stability but in equilibrium with each other The equilibria are to a certain extent influenced by the environment which means that the distribution among the different species of molecules is displaced by changes in the physiological conditions The resultant molecular fluctuation or breathing is fundamental for the many-sided functions of proteins but also has as a consequence that the proteins might pass through molecular conformations which are vulnerable to irreversible denaturation or proteolytic degradation

Summary

From the literature and from the author s own investigations it is illustrated that the albumin molecule *in vivo* has neither its tightest structure nor its maximum stability against denaturation but has a flexible structure and participates in transitions between different conformational states which are determined by the hydrogen ion concentration the temperature and substances bound to the molecule The opening of the albumin structure in question results in an increased probability for intramolecular SS-interchange and other types of denaturation

It is demonstrated that albumin *in vivo* may be converted to its SS-interchanged isomer and that SS-interchanged albumin can be reversed to native albumin This has the consequence that 1/10 of the albumin *in vivo* is present in the SS-interchanged forms The isomers are catabolised considerably faster than native albumin Assuming that the SS-interchange reaction is part of the normal catabolism of albumin it is estimated that at least half of the albumin passes through this isomerization before it is broken down

In connection with other statements in the literature on the turnover of albumin the conclusion is drawn that it is not necessary to presuppose specific mechanisms to handle the degradation of albumin The main part of the catabolism can be explained by an inevitable randomised loss of albumin by denaturation where the final elimination of the denatured albumin takes place by unspecific proteolysis either directly in the extracel ular space or in the cytoplasmatic lysosomes after preceding endocytosis As an additional mechanism for elimination demonstrated by others albumin is continuously exuded from the body especially through the epithelium of the gastrointestinal tract

Summary in Danish

Ud fra litteraturen og egne undersøgelser er det anskueliggjort at albuminmolekylet *in vivo* ikke besidder sin tætteste struktur eller har sin maksimale stabilitet over for denaturering men har en fleksibel struktur og befinder sig på overgange mellem flere forskellige strukturelle tilstande der betinges af hydrogen-jon koncentration temperatur og substanser som kan bindes til molekylet De involverede åbninger af albuminstrukturen medfører en øget sandsynlighed for intramolekylær SS-omgruppering og anden form for denaturering

Det er vist at albumin *in vivo* omdannes til dets SS-omgrupperede isomerer og at SS-omgrupperet albumin kan tilbagedannes til nativt albumin Ca 1/10 af albuminet er *in vivo* til stede i SS-omgrupperet form Disse isomerer kataboliseres betydeligt hurtigere end nativt albumin Ved at antage at SS-omgrupperingen er et led i den normale katabolisme af albumin er det skønnet at mindst halvdelen af albuminet passerer gennem denne isomerisation før det bliver nedbrudt

Sammenholdt med de øvrige angivelser i litteraturen om albumins omsætning kan det konkluderes at det ikke er nødvendigt at forudsætte specifikke mekanismer til nedbrydning af albumin Størsteparten af katabolismen kan forklares ved et uundgåeligt randomiseret tab af albumin gennem denaturering hvor den endelige eliminering af det denaturerede albumin sker ved uspecifik proteolyse enten direkte i extracellulærrummet eller på cytoplasmatiske lysosomer efter forudgående endocytose Tillige sker der som vist gennem andres arbejder et tab ved udsivning fra organismen specielt gennem gastrointestinalepithelet

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TRANSCAPILLARY SOLUTE EXCHANGE IN SKELETAL MUSCLE AFTER INJURY AND DURING SHOCK

By

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Göteborg 1979

ACTA PHYSIOLOGICA SCANDINAVICA
SUPPLEMENTUM 472

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Abstract

DAHLBERG J.B *Transcapillary solute exchange in skeletal muscle after injury and during shock.* Acta physiol. scand. 1979 Suppl. 472. 1–82.

The specific object of the investigation was to study blood flow and blood tissue exchange of small molecules in canine skeletal muscle during hemorrhagic shock, after muscle injury and at normovolemic hemodilution. The effects of regional arterial hypotension and of increased venous pressure were also studied in order to acquire additional information about skeletal blood circulation as it functions in low flow states. The non-isolated hind leg musculature was used throughout the studies except in few experiments, where the hind leg was isolated in order to estimate the capacity of collateral veins. The principal technique employed to study the blood tissue exchange was the single-injection multiple-indicator dilution method. A local clearance method was used simultaneously in some of the experiments on hemorrhagic shock and normovolemic hemodilution. Cr–EDTA was the principal diffusible lipid insoluble indicator for both methods. I-antipyrine was used in the majority of the experiments with the indicator dilution method as lipid soluble test molecule, Fe-siderophilin was the intravascular marker. The capillary blood flow rate in the local clearance method was taken as Xenon clearance. Isotope radioactivities in blood samples were measured in a well-type scintillation counter in muscle depots by external detection. Regional arterial hypotension (50 mm Hg) was produced by partial occlusion f the aorta. Hemorrhagic shock was produced by repeated arterial bleedings to maintain arterial pressure around 50 mm Hg. Muscle injury was produced by a series of hammer strokes on the thigh. Normovolemic hemodilution was achieved by replacing exsanguinated volumes of whole blood with equal amounts of dextran 70 (hematocrit at level 1 25 – 30 %, at level 2 10 – 12 %). Venous pressure was elevated (10 mm Hg) by graded occlusion of the iliac vein. Total blood flow was measured from the timed venous outflow. Values for extraction (E) of diffusible isotopes were used for lipid insoluble test solutes, to calculate the permeability-surface area product (PS). If blood flow (total blood flow or capillary blood flow) is denoted by Q and the natural logarithm by ln, PS = $-Q\ln(1-E)$. The evaluation of the results was promoted by a computerized statistical analysis of the data, including the assembled data from the control i.e. the basal states. The extraction of I-antipyrine, 0.79 was independent of changes in blood flow rate, induced in the different activated states. PS found (for Cr–EDTA) to change with flow decreased as a result of severe hemorrhage; this was the case for all lipid insoluble indicators (^{125}I ^{131}I, ^{51}Cr–EDTA) used with the indicator dilution method. PS(Cr–EDTA) was reduced by 50 % after two hours of hemorrhagic hypotension. The magnitude of this decrease in PS was the same when estimated by the local clearance method. F llowing injury total blood flow either increased or decreased PS(Cr–EDTA) decreased. The increase in plasma flow seen at both levels of normovolemic hemodilution (dextran 70) and registered by both the indicator dilution and the local clearance method was accompanied by an increase in PS(Cr–EDTA) when the measurements were made by the indicator dilution method, no change in PS(C –EDTA) was registered when measured simultaneously by the local clearance method. Following graded occlusion of the iliac vein, a part of the venous return was diverted into collateral blood vessels leading from the hindleg; the elevation of venous pressure did not appear to influence the PS value for Cr–EDTA. It was concluded that, when the blood flow in the muscle decreases as a consequence f partial occlusion of the aorta, severe hemorrhage or injury the PS values for lipid insoluble solutes also decrease. It was also concluded that an increase in flow, as seen during normovolemic hemodilution with dextran 70 may be accompanied by an increase in PS but also, as seen after injury by a PS reduction. Finally it was concluded that elevation of venous pressure does not affect the passage across the capillary wall of small solutes like Cr–EDTA.

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CONTENTS

INTRODUCTION

The vascular bed in skeletal muscle is of very great importance in the regulation of the general circulation and for the satisfaction of increased metabolic demands occurring, e.g. during muscular work or in disease

Much data have been accumulated over the latest century about the basic morphologic al and physiological properties of this vascularity The methods which have been employ ed to study the capillary blood circulation in skeletal muscle have rarely been free from difficulties Some difficulties have arisen as a result of the large variability of peripheral blood flow and its relation to the variety of different properties of the capillary wall and its adjunctive tissue. They have been evident, in particular when diffusion exchange across the capillary wall has been studied in whole organs.

The degree of variation which characterizes many events in a vascular bed may often be raised in *pathological* states. This could add to the variety of difficulties involved when the circulatory response to *injury* is to be studied and could help to explain why so much uncertainty remains about capillary permeability and blood-tissue exchange e.g. in skeletal muscle after injury and during various kinds of shock.

AIM OF THE PRESENT STUDY

The purpose of the present investigation was to study the blood flow response and the blood tissue exchange of small molecules in dog skeletal musculature during shock. The single-injection multiple-indicator dilution method was used to measure the transcapillary solute flow from *blood to tissue* in some conditions of surgical interest, where the skeletal blood circulation is greatly affected. The technique was employed for studies on hemorrhagic shock, tissue trauma and hemodilution. In the hemodilution experiments no deliberate attempt was made to produce a shock-like condition in the animal prior to the hemodilution.

To elucidate such aspects as hypotension in shock and venous stasis in trauma additional studies were performed on the effects of regional arterial hypotension and of regionally increased venous pressure

A local clearance method was used simultaneously in the series of experiments on hemorrhage shock and on hemodilution to measure *tissue to blood* transport.

Throughout the study it was assumed that total blood flow from the hind leg musculature which was under investigation, was represented by the flow from the iliac vein and, further that this blood flow represented the total capillary blood flow through the musculature The first assumption was tested by comparing arterial inflow and venous out flow in some experiments at normal and increased venous pressure.

BACKGROUND

A. Regulation of peripheral circulation and of solute and water flows across the capillary wall

The mechanisms by which the peripheral circulation is regulated are sensitive both to the influence of locally situated control systems within the peripheral tissue and blood vessel walls and to centrally generated nervous and humoral control The interaction of these control systems is not only regulating the peripheral blood perfusion but also the ex change processes between blood and tissue (e.g. Renkin 1964 Mellander 1960, 1971) These processes are relatively more sensitive to influence of the local control mechanisms because of the particular sensitivity of the muscle tone of the precapillary sphincters to changes in local metabolism and blood pressure variations (Mellander and Johansson 1968 Mellander 1970). The nervous, mainly vasoconstrictor influence on the capillary exchange area on the other hand is less sustainable but of particular importance for fluid absorption by its decreasing effect on the capillary pressure (Cobbold et al 1963 Öberg 1964)

The diffusion flow of water soluble (lipid-insoluble) solutes, mainly confined to pore pathways depends on the capillary permeability(P)-surface area(S) product(PS) and on concentration gradients across the capillary wall The magnitude and importance of convection exchange of solutes across the capillary wall are much debated. It seems to increase with molecular size so that for large solute molecules it may even predominate over dissipative transport especially at abnormally high rates of net volume flow across the capillary wall (Renkin et al 1977a, b Rippe et al 1979).

In contrast small lipid-soluble molecules, such as Xenon and antipyrine will meet practically no barrier for transport at the capillary wall

The exchange of water is basically regulated by the capillary hydrostatic pressure and the plasma protein osmotic pressure (Starling 1896 Landis 1927). The capillary pressure depending on the post to precapillary resistance ratio tends to increase in vasodilatation (Pappenheimer and Soto-Rivera 1948). Water is crossing the capillary wall via pathways through the endothelial cell membrane and via intercellular pathways, both small and large (e.g. Curry et al 1976 Renkin and Curry 1978).

The presence of plasma proteins is also necessary for the maintenance of a normal capillary permeability (e.g. Danielli and Stock 1944 Levick and Michel 1970, Curry et al 1973 Mason et al 1973 1977)

B Methods for measuring transcapillary exchange

In order to analyse the effects of capillary blood flow on diffusion transport of solutes

models of the capillary or the capillary bed have been devised (Krogh 1919 Kety 19... 1957 Renkin 1959 cf Reviews and Models) The construction of such models has necessitated several assumptions concerning the structure and function of the capillary bed. In the single capillary model, which was originally used to formulate a relationship between the product of capillary permeability and surface area (PS) on one hand and ... flow and solute fractional extraction (E) on the other

$$PS = -Q \ln(1-E) \qquad \text{(Renkin ...)}$$

It is assumed that the permeability along the capillary is uniform and that there is a progressive concentration fall of a solute moving along the capillary Any diffusion gradients or permeability barriers outside the capillary are not accounted for (cf. Crone and ... 1970) When the formula is used for studies on an entire capillary bed rather than a single capillary one has to presuppose that there is uniformity between all the capillaries with respect to their individual exchange/blood flow ratio (or ps/q ratio) The value of PS and PS/Q calculated from extraction and flow for the whole vascular bed where extraction (E) will be a flow weighted mean value of extractions [1 − exp(−ps/q)] in different parts of the capillary bed may hide complex patterns of distribution of ps q ...

1 The single-injection indicator-dilution method

This method originally devised by Chinard and colleagues (1955) is a ... the technique of Stewart (1897 1921a, b) and Hamilton (Kinsman et al ... et al. 1932) for measuring blood flow It has been extensively employed ... various organs and tissues (see separate list of references). With few exce... method has only been applied in studies on normal physiological states ... been done in skeletal muscle during injury or disturbed circulation ...

The technique is based on the measurement of the fractional extrac... substance from the blood into the tissue during the first passage throug... bed. The extraction is derived from concentrations at the venous outle... tracers as fractions of an indiffusible tracer injected simultaneously ... bolus. With the single capillary model, it is assumed that there is an exp... cular concentration fall of the diffusible indicator along the capillary ... that this indicator is dispersed intravascularly to the same extent as the ... dicator

$$C_v/C_a = \exp(-PS/Q)$$

C_v is the venous concentration of the diffusible indicator C_a the ... concentration of the indiffusible indicator P is the capillary perme... pillary area available for indicator diffusion. Q is the blood flow throug... assumed to be equal to the total blood flow through the organ. The ... indicators are uniformly dispersed intravascularly is a simplification ...

of error since it is known that the indicator molecules of different molecular weights disperse differently (Taylor 1954).

The fractional extraction (E) of the diffusible indicator is $(C_a - C_v)$ $(C_a - C_i)$ The tissue concentration of the tested solute is supposed to be so low that no back-diffusion takes place during the early stage of solute passage This is the case if C_t the tissue concentration of the diffusible indicator is zero during the early phase of the indicator passage through the organ (Crone 1963 Martin de Julián and Yudilevich 1964 Yudilevich et al. 1968). PS may then be calculated from the equation PS = Qln(1−E) without correction for back-diffusion

More extensive calculations, taking into account the whole period of exchange after injection, including back-diffusion, have been applied, e.g. by Goresky and Bassingthwaighte and their colleagues (1963 1970 1970, 1974 cf Methods and Theory). These methods (see e.g. Guller et al 1975) are beyond the scope of the present study

In order to avoid the difficulties concerning non-uniformity of extraction values among the capillaries within the vascular bed and flow-limitation of transcapillary exchange, the method is often employed at high rates of blood flow which, in practice implies a PS/Q ratio less than 0.5 (Goresky et al. 1970, Bassingthwaighte 1974 Guller et al. 1975 Rippe 1978). As mentioned earlier the formula is based on the assumption that the fractional extraction is the same in all the capillaries of the capillary bed.

The transcapillary passage of the lipid soluble antipyrine is rapid in relation to blood flow Its extraction is therefore expected to be constant at various flow levels, indicating a flow-limited clearance (Renkin 1952, 1953).

2. The local tissue clearance method

This modification of the tissue clearance method (Kety 1949) was devised to study the capillary permeability-surface area product (PS) by simultaneous clearances of an inert gas and a hydrophilic ion (Gosselin 1967 Lassen and Trap-Jensen 1968 Appelgren and Lewis 1968 Strandell and Shepherd 1968). It is assumed that no diffusion gradients in the interstitial space will develop at any time during the washout period and that there is uniformity of the capillary bed.

The method is based on the assumption that *the fractional washout rate (dA/dt) x (1/A)* from the tissue interstitium is constant both for the hydrophilic small molecule (as Cr−EDTA) and the lipophilic gas (as Xenon) for a period following the simultaneous local injection into the tissue When the local tissue clearance method is employed it is also necessary to assume that the interstitial volume constitutes a specified percentage of the tissue and that there is a uniformity in the capillary bed. Osmotic effects of the injectate are as in the indicator dilution method considered to be low enough to be disregarded.

Xenon, an inert gas, is expected to equilibrate with blood during its passage through the tissue giving a Xenon clearance equalling the blood flow Q.

Calculation of clearance and extraction is made according to the equations

$$dA/dt = -k \times A$$

$$C = \lambda \times 100 \times k \qquad ml/min/100\ g\ tissue$$

$$E = C/Q = 1 - \exp(-PS/Q)$$

where k is the fractional washout rate in min^{-1} and λ the tissue-blood partition coefficient. A is the (relative) counts per minute of the residual radioactive activity in the indicator depot.

The extraction of the hydrophilic molecule is the ratio of the clearance values of this substance to that of the gas, if the gas clearance value indicates blood flow (E = C/Q). For some molecules diffusion gradients are likely to arise in the interstitium, although such effects depend on the molecular size of the hydrophilic substance and seem to be minimized for certain substances, e.g. ^{51}Cr–EDTA (Mw = 341) (Lassen and Trap-Jensen 1970).

C Some current concepts on blood tissue exchange in states of disturbed circulation

1 Regional arterial hypotension

Renkin (1959) found a systematic variation in measured permeability-surface area product (PS) with changes in blood flow and vascular resistance indicating that the capillary circulation is not uniform and that observed PS values principally refer to transcapillatory exchange in well-perfused blood vessels.

Renkin s observations have been confirmed in several studies on blood tissue exchange (Trap-Jensen and Lassen 1970, Appelgren 1972, Linde, Chisholm and Rosell 1974 Paaske 1977 Rippe Kamiya and Folkow 1978). This systematic relationship between observed PS values and rates of blood flow was seen even when the vascular bed is dilated. It therefore seems likely that stagnancy of capillary blood flow may have similar effects on blood tissue diffusion exchange as actual closing or corpuscular obstruction of parts of the capillary bed.

2. Hemorrhage

Acute hypovolemia elicits a great variety of reactions concerning the blood circulation. Some of the reactions, such as those affecting vascular adaptation and fluid transfers between tissue compartments, are comparatively well known, both in a qualitative and in a quantitative respect (see Reviews). Some of the changes seen in hemorrhagic shock may be caused solely by the reduction of blood flow whereas others apparently occur as a consequence of an altered distribution of capillary blood flow (Appelgren 1972). The redistribution of blood flow does not seem to be caused only by compensatory reflex mech-

anisms, responding to decreased blood flow and perfusion pressure (Mellander and Lewis 1963 Chien 1967 1969 Mellander and Öberg 1967 Järhult et al. 1972, Järhult 1973 1975). It also appears to be a less adaptive response, due to the early development, in shock, of a large scale capillary imperviousness or stasis, mainly caused by obstructing blood corpuscles and other rheological factors.

Data indicating such changes have been obtained in studies on the capillary filtration coefficient, giving the impression that extensive areas of the capillary bed in muscle become obstructed after hemorrhage (Baeckström et al. 1971).

The transport of solutes from the interstitial fluid, expressed as capillary diffusion capacity (PS), to the blood has also been found to become greatly reduced during hemorrhagic shock (Appelgren 1972). At perfusion pressures above 60 mm Hg, the reduction was equivalent to a 50 % decrease of the permeability surface area product (PS) in comparison with control values at similar perfusion pressures. This finding was interpreted as being due to a reduction of the number of perfused capillaries. An additional reversible, reduction of PS was seen at variation of perfusion pressures within the 20 – 60 mm Hg range coinciding with a similar kind of response in PS of controls. It was proposed that such a PS reduction in shock at low perfusion levels can be explained by an increasing heterogeneity of the residual capillary blood flow

Injury

Landis (1934) described the complex response that local injury initiates: vasodilatation, rise in capillary blood pressure first increased, then decreased blood flow increased endothelial permeability and finally stasis. In their review in Handbook of Physiology (1963) Landis and Pappenheimer knowing that diffusion rates had not been previously measured when effects of severe injury were studied, presumed one should expect an increased net diffusion of molecules across the capillary wall as long as blood flow continues (at an unchanged or even increased rate) but that the effectiveness of diffusion, in exchanges of substances would decline as flow decreases or ceases, since capillary exchange would then become flow limited. Landis (1927) found, when studying the increased permeability in injured capillaries, that increase of the capillary blood pressure was a more consistent response to injury than vasodilatation.

Regional contusion of the dog's skeletal musculature has often been seen to cause an immediate local vasodilatation (Liu 1968 Lewis and Lim 1970, Sandegård 1974). This hyperaemia is assumed to have certain characteristics in common with metabolic vasodilatation (exercise or functional hyperaemia and reactive hyperaemia following temporary arterial occlusion) in that the myogenic tone of the precapillary resistance vessels is presumed to diminish by the influence of locally accumulated metabolites. In reactive hyperaemia the myogenic tone is also influenced by perfusion pressure change since blood pressure reduction, like tissue hyperosmolality inhibits the myogenic pacemaker

activity in the walls of precapillary resistance vessels. Locally accumulated metabolites during muscle exercise or at decreased blood flow produce vasodilatation by similar mechanisms tissue hyperosmolality hypoxia and extracellular potassium are all known to reduce the tone in both precapillary resistance vessels and sphincters (e.g. Johnson 1974).

4 Hemodilution

Reduction of the plasma volume and disturbance of the capillary blood flow are common features in various states of shock. Plasma substitutes, often primarily administered to re store the intravascular volume of blood plasma, may also influence the distribution of the capillary blood flow and, in consequence of that, the blood-tissue exchange of water and water soluble molecules.

Solutions of dextran 40 and dextran 70 have been shown, in particular to possess bene ficial properties as plasma substitutes in shock (Gelin 1962a, b 1973 Lim et al. 1968 Dawidson et al. 1979b c). The blood tissue diffusion exchange of small hydrophilic molecules has been found to improve, in shock, after administration of dextran 40 (Appelgren 1972) Comparative studies are currently under progress to examine the properties of various kinds of infusates, including dextran 40 and dextran 70, with respect to their influence on transcapillary solute exchange and tissue O_2 tension in skeletal muscle during shock (Dawidson et al. 1979a). The disturbance of capillary circulation, which is seen to follow arterial hypotension (cf. Järhult and Mellander 1974) appears to be markedly alleviated by dextran 40 (Appelgren 1972).

The mentioned dextran solutions seem to counteract the non-uniformity and obstruction of capillary blood flow which follow hypotension and shock, thus establishing a larger "effective" capillary surface area available for diffusion transport. The exact mechanisms, by which such an improvement is achieved are not, as yet, fully understood.

5 Increased venous pressure

It could be argued that an extensive preparation procedure, apart from having possible side-effects on the distribution of capillary blood flow may also affect the blood flow through the major pelvic veins by exposing these vessels to an increased external pressure. The effect of an increased venous pressure on blood flow and transcapillary solute exchange would depend on

a) the opening of alternative routes for the blood returning from the hind leg, e.g. collateral veins,

b) possible changes of total capillary blood flow and capillary surface area and

c) fluid movements from blood to tissue induced by an increased capillary pressure

It has only occasionally been proposed that solvent drag can account for any consider able part of the transport of small lipid insoluble molecules (Åberg 1973). Pappenheimer et al. (1951) calculated the relations between diffusion and filtration transfer for various

[illegible]

[illegible] effect of surgical preparation

[illegible] the [illegible] in tissue [illegible] [illegible] it is generally [illegible] that [illegible] of tissue [illegible] which are [illegible] The distribution of capillary blood flow e.g. seems to be more [illegible] blood flow [illegible] a considerable reduction of [illegible] and [illegible] of the [illegible] PS [illegible] model [illegible] effects of the surgical preparation should be kept in mind when the magnitudes of [illegible] PS and PS changes induced are to be [illegible]

MATERIAL AND METHODS

The experiments were carried out on 78 adult mongrel dogs of both sexes (9.5 to 28 kg). Splenectomy was performed at the initial stage of the experiments on hemodilution Eleven experiments were discarded due to untimely animal deaths or to technical errors. One dog was exclusively used to test the experimental set-up and the isotope injection and blood-sampling techniques. Three dogs were used entirely for comparative blood-flow measurements.

Experimental procedures

The dogs were anaesthetized with sodium pentobarbital intravenously Small supplementary doses were given as required. The airway was secured by an endotracheal tube In the series of experiments on trauma the breathing was further supported and controlled by a respirator The animals were not heparinized. The vascular bed of the hind leg musculature was not vasodilated.

Infusates

Saline was slowly infused intravenously throughout the experiments via a catheter to one jugular vein

Six per cent dextran 70 (Macrodex® Mw about 70000 supplied by Pharmacia, Sweden) was used to achieve normovolemic hemodilution.

Pressure and flow measurements

Arterial blood pressure was measured by a mercury manometer via a catheter from one carotid artery and also in the experiments on regional arterial hypotension, from the sacral artery

Venous blood pressure was measured by a water manometer via a wide bore siliconized plastic T-tube placed in one iliac vein.

The venous return was diverted through the T tube for serial blood sampling (see indicator-dilution technique) and was timed in order to measure blood flow The weight of the hind leg was registered at the end of each experiment f r conversion of blood flow to ml/min/100 g.

Regional arterial blood flow was directly measured in a few experiments in order to compare arterial blood flow into the hind leg with venous outflow (for a detailed description see Chapter 9) An electromagnetic flow meter (Nycotron, Oslo Norway) was used with the probes placed on the external iliac artery proximal to the deep femoral artery

Isotopes

I For indicator-dilution method

Diffusible

Water soluble lipid insoluble extracellularly distributed

a) ^{51}Cr–EDTA. T 1/2 (^{51}Cr) 27.8 d. γ-energy (^{51}Cr) 0.320 MeV Mw 341

b) ^{125}Iodide T 1/2 60 d. γ-energy 0.035 MeV

c) ^{131}Iodide T 1/2 8.04 d. γ-energy 0.364 MeV

Lipid soluble intracellularly distributed

^{125}I antipyrine.

Monthly prepared and dispatched, free iodide less than 5 %. Mw 312.2.

Non-diffusible

a) ^{59}Fe-siderophilin. T 1/2 (^{59}Fe) 45 d. γ-energy (^{59}Fe) 1.10 MeV

Prepared by mixing $^{59}FeCl_3$ with dog's plasma.

b) ^{125}I–RIHSA Free iodide less than 5 %

II. For local clearance method

^{51}Cr–EDTA (see I).

^{133}Xenon. T 1/2 5.3 d. γ-energy 0.081 MeV Dissolved in saline solution. 0.1 ml injected. Specific activity 2 – 10 Ci/cm^3 Xe at stp

Measurement of radioactivity

The radioactivity of blood samples was measured in a well type scintillation counter with a γ-spectrometer The three isotopes, ^{125}I ^{51}Cr and ^{59}Fe were measured on three different channels. Corrections for background and for interferences between the various isotopes were made The radioactivity from isotope depots in muscle tissue was measured by a single scintillation counter with a two-channel spectrometer with ratemeters and recorders.

Hematocrit was measured by microcapillary centrifugation and used in the calculation of tissue-blood partition coefficients of Xenon (Andersen and Ladefoged 1967) and of ^{51}Cr–EDTA (Appelgren 1972b).

Experimental procedure for the indicator techniques

I The single injection multiple indicator dilution method

In the present study the indicator dilution method was used for meas

of the blood tissue exchange in a non-vasodilated vascular bed. Following laparotomy the vasculature to one hind leg of the dog was freed from above the bifurcation of the aorta to the inguinal ligament. The blood circulation of the paw was excluded by a ligature around the ankle A catheter was introduced into the iliac artery via a small sidebranch. A mixture was prepared of dog plasma and appropriate amounts of isotopes (approx. 150 – 250 μCi for ^{51}Cr–EDTA, 100 – 150 μCi for ^{125}I-antipyrine 15 – 20 μCi for $^{59}FeCl_3$). One ml of this mixture was used for each injection which was rapidly made into the artery with a syringe The blood flow from the iliac vein was, via a wide-bore catheter collected in vials, placed in a fractional collector (Fig. 1). Collection time varied between 20 seconds and 2 minutes depending on flow rate. The amount of the various isotopes in the injectate was measured at the end of each experiment and used to normalize the time-concentration curves that were constructed for each isotope. Normalizing was achieved by calculating the concentration of various isotopes in the fractional blood samples, expressing the concentration as per cent of given amount in the injectate per gram blood (Fig. 2). Radioactive background activity was measured from early blood samples collected before the injectate started to reach the collector $^{59}FeCl_3$ was used in all experiments as a vascular marker It was assumed that this isotope when binding irreversibly to siderophilin, functions as an indiffusible indicator (Yudilevich and Martin de Julián 1965).

The diffusible isotopes used were iodide ion (^{131}I or ^{125}I) or, most commonly ^{51}Cr–EDTA together with ^{125}I-antipyrine. ^{125}I–RIHSA was used in two experiments for comparison with ^{59}Fe-siderophilin.

Extractions of diffusible isotopes were calculated as $E = (C_{nondiff} - C_{diff})\ (C_{nondiff})$, where $C_{nondiff}$ stands for concentration of ^{59}Fe-siderophilin and C_{diff} for either ^{51}Cr–EDTA, ^{125} or ^{131}I or ^{125}I antipyrine, after standardization of isotope concentrations for injected amount of the isotopes in the arterial bolus (Fig. 2).

2. The local clearance method

^{133}Xenon and ^{51}Cr–EDTA were mixed and 0.1 ml of the mixture slowly injected into the muscle The injection needle remained in the muscle for 30 seconds following the injection. The radioactivities at the injection site were counted by a scintillation detector and recorded. After corrections for background activity (^{51}Cr–EDTA) and interference (^{133}Xe) the radioactivities as counts per minute were plotted semilogarithmically against time k-values were calculated from t 1/2 of the monoexponential part of the curve Clearances were calculated from k-values and the partition coefficients. Extraction of ^{51}Cr–EDTA was estimated as the ratio between the clearances of this substance and ^{133}Xe and subsequently used in the formula $E = (C/Q =)1 - \exp(-PS/Q)$.

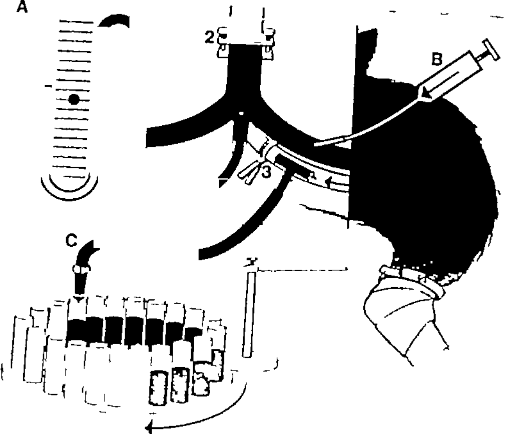


Fig. 1 Diagram of the experimental set-up for indicator dilution tudie on tl during regional arterial hypotension produced by compression of the aorta t return in the iliac vein (occlud d at 3) is sampled in vi placed on rotati g a constrictor (1).

ring arterial pressure rators. C. The total venous u al art f the hind leg is excluded by

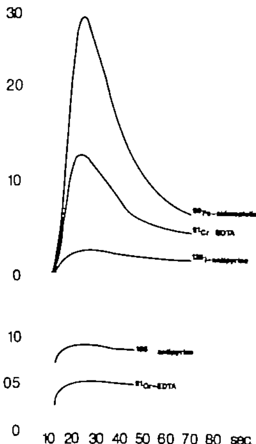


Fig. 2. Results of single injection of 1 ml plasma containing ^{59}Fe-siderophilin, ^{51}Cr–EDTA and ^{131}I-antipyrine. Venous concentration curves (above) per cent of injected amount of isotope per gram blood in each sample. The injection was made t time zero. From the data obtained for each blood sample, *extraction* values for the two diffusible indicators were calculated (below). In this experiment plateau levels for extraction were achieved very late in the upslope curve for the non-diffusible indicator. Note that initial extraction values are lower than the plateau values.

Presentation of the relations between extraction (E) blood flow (Q) and permeability-surface area product (PS)

The main purpose of the present investigation was to study blood tissue exchange by the indicator dilution method in some experimental situations where arterial or venous blood pressure was changed or where capillary blood perfusion was changed as a consequence of shock or trauma. The experiments were not designed to yield any direct information about capillary permeability but rather to permit observation of PS changes, which, in relation to blood flow could possibly be used for comparison between different groups of experiments.

f und convenient to plot extraction values (Cr–EDTA) k ound of theoretical PS curves showing the interrelation ı constant PS values in accordance with the equation p(S Tl seen in Fig. 3 In this way any observed change of extraction

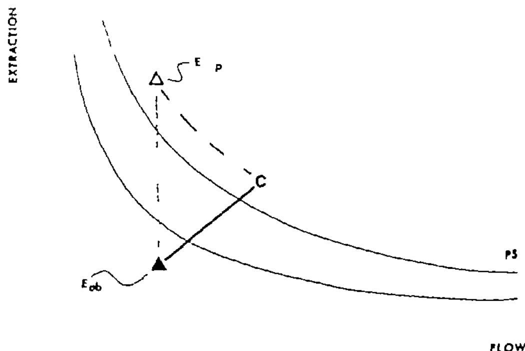


Fig. 3 Schematic drawing illu trating how a change in extraction from control (C) can be estimated in relation to various values of the PS product [background curves according to $E = 1 - \exp(-PS/Q)$]. Mathematically this change may be expressed as a ratio between the extraction value obtained (E_{obs}) and the extraction value expected t result t an unchanged value of PS and t a similar change in flow (E_{exp}).

could be visually compared to the one expected with the change of Q at constant PS. Each figure containing data from a group of experiments (Chapters 5 6 8), is accompanied by a figure illustrating mean changes (in per cent) in PS and blood flow for paired comparisons with the control set to 100 % for PS and blood flow

The extraction of antipyrine is only presented in the tables since as will be shown the flow dependence of extraction was small or non-existing within the flow range studied.

Statistical methods

The objectives of the stati * for iodide Cr–EDTA and antipyrine blood flow es for (iodide Cr– EDTA). The PS values f TDTA od flow (Q)

and extraction (E) as

$$PS = -Q\ln(1 - E)$$

where ln denotes the natural logarithm.

These parameters and their interrelationships were studied separately by a computerized statistical analysis comprising data from the control state and they were also analysed in relation to some variables, which may be applicable in the assessment of the condition of the experimental animal (see Chapter 4).

To evaluate the response in the various parameters following arterial hypotension haemorrhage etc. mean values and the limits for the mean plus and minus one standard deviation were computed *for all* available pairs of observations. The changes were also calculated in per cent (except for antipyrine extraction), giving mean values and a 99 % confidence limit. The change is considered to be statistically significant at the 1 % level if both confidence limits are either less (decrease) or larger (increase) than 100 %. For antipyrine extraction, changes are statistically significant if both limits are either positive or negative

CHAPTER 3

ANALYSIS OF VENO-CONCENTRATION AND EXTRACTION CURVES (INDICATOR DILUTION METHOD)

Since the indicator dilution method has been employed throughout the present studies, it was considered to be of interest to present some complete curves, illustrating this method at different blood flow levels and, in turn, with different Cr–EDTA extractions at these flow levels. Each E/Q value of the diffusible indicators (Cr–EDTA, iodide and I-antipyrine) presented in the following chapters was estimated from one or two (in con trol and after hemodilution) sets of such curves. Each set of curves represents a single injection of isotopes and the serial blood samples which were subsequently collected (Fig. 2).

Results

There was a considerable variation of the configuration of veno-concentration curves. The time for blood sampling after each intra-arterial injection of indicators was adjusted to a length appropriate for prevailing blood flow rate. At very *low* blood flows, concentration curves were occasionally obtained where peak concentration of the reference indicator was not reached within the sampling time Only very rarely was blood sampling, at relatively *high* blood flows, terminated before peak concentration had been reached. The configuration of the veno-concentration curves, however was not only depending on the blood flow rate

Within the low blood flow range, curves were obtained, which displayed an individual variability This is exemplified in Fig. 4 where the curves represent three different measurements from two experiments. In the upper part of the graphs, the appearance time is seen to vary by 10 seconds and the time to reach peak concentration from very short time to more than 15 seconds despite comparable values of total blood flow The corresponding extraction curves (lower part of graphs) display a plateau of extraction values, which corresponds to parts of the veno-concentration curves before or around the peak. The E_{Cr} values vary between .50 and 13 Also at comparable, higher blood flows, variations of time-concentration curve configuration and extraction levels were found (Fig. 5).

Comments

It was often found that time-concentration curves had not been recorded long enough to display a curve peak or a down-slope Nevertheless, extraction values forming a plateau were obtained from the existing part of the curve Attempts to measure the blood flow by calculation of the area under the curve after extrapolation from the early down-slope of the curve (for indiffusible tracer) were not made due to the scarcity of adequate

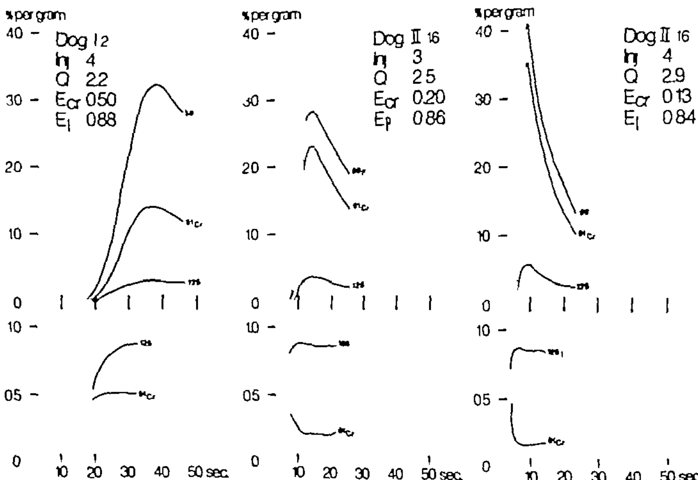


Fig. 4 Various concentration (or indicator dilution) curves for [59]Fe-siderophilin, [51]Cr–EDTA and I-antipyrine (above). Corresponding extraction curves (for C –EDTA and I-antipyrine) are seen below each set of dilution curves. Data are presented in accordance with the description given in Fig. 2 The two sets of curves t the left refer to an experiment on the effects of regional arterial hypotension the others to measurements med in period f hemorrhagic hypotension. The curves have been selected in order to illustrate the variation in the shape of curves obtained within range of comparably low blood flow levels. The extraction value for Cr–EDTA is seen to vary t large extent.

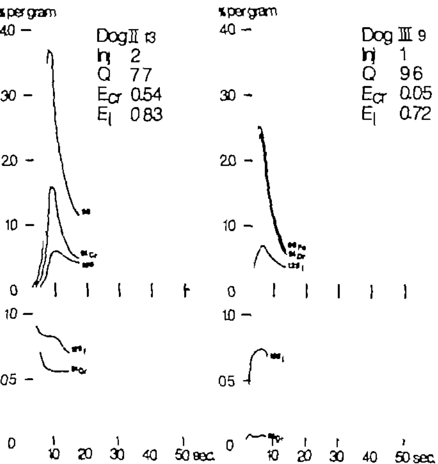


Fig. 5 Indicator dilution curves for ^{59}Fe-siderophilin, ^{51}Cr–EDTA and ^{125}I-antipyrine (above). Extraction curves, for Cr-EDTA and I-antipyrine, calculated from the data above, are seen below each set of venous concentration curves. As in Fig. 4 graphs have been selected so as to illustrate the variation in the configuration of curves obtained, in this case t comparable, higher rates f blood flow N to the different plasma levels for the extraction of Cr EDTA.

curves. For the same reason mean transit time was not calculated, the error introduced by extrapolation even in more "complete" curves was reckoned to be too large (cf. Ripp and Stage 1978)

Summary

Some indicator dilution curves have been presented representing three isotopes at different rates of blood flow The curves illustrate

1) that the ^{125}I-antipyrine extraction was approx. 0.8 and independent of blood flow rate and of the ^{51}Cr–EDTA extraction,

2) that, at two different levels of perfusion rate (2 – 3 and 8 – 10 ml/min/100 g resp.), ^{51}Cr–EDTA extraction might vary considerably at different injections,

3) that for each isotope there was a plateau of consecutive extraction values soon after the appearance of the isotope in the venous effluent. The level of this plateau was used as extraction value for the indicator

In addition it was concluded

4) that the configuration of the dilution curves did not permit any regular estimation of blood flow (from the dilution curve of the indiffusible indicator). The limited sampling time made available curves inadequate for estimation of mean transit time

CHAPTER 4

DESCRIPTION OF THE CONTROL STATE (INDICATOR DILUTION METHOD)

The variability described in the previous chapter is amply illustrated in the series of E_{Cr}/Q diagrams which are to be presented in the following chapters. It was seen both during the control state and during the activated states. Due to the variability of E/Q a separate analysis of the control state was considered to be of interest.

In order to ascertain whether any justification could be found to divide the group of control data, e.g. according to level of PS (Cr–EDTA), the relations between the parameters and different background variables concerning the condition of the experimental animal were statistically analysed. It could be argued that some animals had, in terms of reaction to preparation trauma, suffered a more profound disturbance of their peripheral circulation than others (Appelgren 1972). If this were to be the case, the mode of reaction to different experimental procedures after the control period may possibly vary accordingly

Methods

The control values for extraction (E) of the diffusible, water-soluble indicator (usually ^{51}Cr–EDTA) extraction of ^{125}I-antipyrine, PS (^{51}Cr–EDTA) and blood flow (Q) were subjected to a computerized statistical analysis in order to describe the control state in 63 dogs. In each experiment one or *two* control measurements were used in form of their mean value The measurements (in Chapters 5 – 9) of Q and of E and PS for the lipid insoluble indicators (iodide ion, ^{51}Cr–EDTA) were subjected to a logarithmic transformation since their distributions were not normal but positively skewed. The means presented are the geometric means and the description, in the tables, of the dispersion in terms of the mean plus and minus one standard deviation is not symmetric around the mean. For the I-antipyrine measurements only E is considered and these measurements have not been transformed.

Results

A large scattering of the Q E_{Cr} and consequently PS_{Cr} values was found, mainly reflecting an individual variability of the vascular bed in the participating animals. The distributions of Q PS_{Cr} and E_{Ap} are presented in Fig. 6. The mean values were within the range to be expected in a resting musculature Q = 4.6 ml/min/100 g (MV + SD 6 9 MV – SD 3 1) E_{Cr} = 0.20 (MV + SD 0.44 MV – SD 0.09) and PS_{Cr} = 1 15 ml/min/100 g (MV + SD 2.68 MV – SD 0 49) The mean E_{Ap} was 79 (SD 08). Some relations between the

parameters were evaluated

1) E_{Cr} vs Q correlation −.29 (rank correlation −.33)

2) $\ln(1-E_{Cr})$ vs 1/Q correlation −.20 (rank correlation −.33)

3) PS_{Cr} vs Q correlation .25 (rank correlation .26)

4) E_{Ap} vs Q correlation .21

The first two of these four correlations were statistically significant.

No statistical justification could be found to treat the group of control data as more than one homogeneous set of values. No significant correlations were found to exist between any of the parameters (Q, E, PS) and the following variables: body weight, rectal temperature, arterial blood pressure, hematocrit and preparation time.

Comment. The use of the control state for the evaluation of the activated state

To illustrate the response produced by regional arterial hypotension, hemorrhage and hemodilution, the data are presented in

a) tables showing mean values and limits for the mean, and

b) tables showing mean changes (in per cent) and their confidence limits for paired comparison of measured values for $PS_{Cr-EDTA}$ and Q in the control state (most often mean of two values) and corresponding PS/Q pairs in the activated state.

For the same indicators (iodide ions, ^{51}Cr−EDTA) changes in per cent are also presented for observed extraction in relation to expected extraction at unchanged PS (with identical change of flow), expressed as their ratio (Fig. 3).

This parameter, $E_{OBS}/E_{EXP} \times 100$, should give results very similar to those obtained for the change in PS, at least at low extractions. In any comparison all available pairs of observations have been used.

Changes of extraction for the lipid soluble indicator, l-antipyrine, are expressed as absolute differences, considering the small and flow independent changes from the control to the activated state.

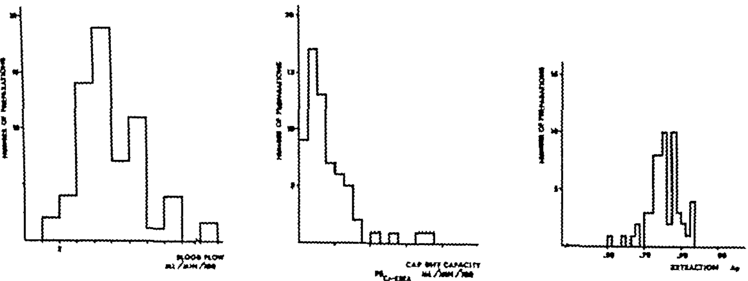


Fig. 6. Histograms of values for blood flow capillary diffusion capacity (PS_C EDTA) and extraction of I-antipyrine. The determinations were made when there were no obvious indications of any induced injury i.e. the measurements were all made in the control state. It can readily be seen that the values for PS_C EDTA have a skewed distribution, which is also the case for the distribution of blood flow. The mean values of blood flow and PS_C –EDTA given in the text with asymmetric standard deviations, due to the skewed distributions.

trated in Fig. 7 (right panel).

The mean change of ^{125}I-antipyrine extraction was 0 03 (−0 03 0 09).

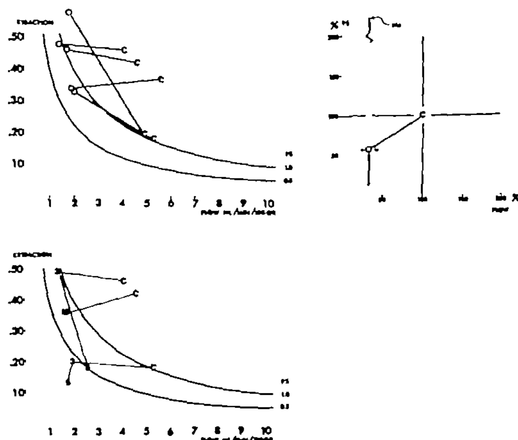


Fig. 7 Single-injection multiple-indicator dilution method. Extraction/flow diagrams to the left, changes in PS and flow (in per cent) to the right. The changes in extraction and PS for Cr–EDTA, induced by *short-term regional arterial hypotension* in five experiments (above left). An open circle indicates the individual value in relation to its control (C).

To the right, the changes in PS and flow [paired comparisons with control (C)] have been expressed in per cent with the control value set to 100 %. The open circle indicates mean values of PS and Q; 99 % confidence limits for PS and Q have been drawn. At reduced perfusion pressure (approx. 50 mm Hg) both blood flow and PS are seen to decrease. The change of PS, however is statistically insignificant (upper right).

In three of the experiments (below), extraction values were also obtained after three (3) and five (5) hours during a period of *prolonged hypotension*.

	Control			Hypotension		
	N	Mean	Mean±SD	N	Mean	Mean±SD
^{51}Cr–EDTA						
Blood flow	5	5 4	4.3–6 7	5	1 7	1.5–2.0
Extraction	5	0.30	0 19–0.47	5	0 43	0.34–0.54
PS	5	1 74	0.95–3 17	5	0.99	0.75–1.31
^{125}I-antipyrine						
Extraction	5	0.84	0.80–0.88	5	0.87	0.82–0.92

Table I. Mean blood flow extraction and permeability surface area values before and after reduction of perfusion pressure.

	Effect %	Conf. limits %
^{51}Cr–EDTA		
Blood flow	33	(24–45)
Extraction	142	(51–399)
PS	57	(11–292)
Observed/expected extraction	61	(31–121)
^{125}I-antipyrine		
Extraction (absolute difference)	0 03	(–0.03 0.09)

Table II. Change of extraction, blood flow and permeability surface area following reduction of perfusion pressure.

Comments on results

In the present study PS (Cr–EDTA) and blood flow were seen to decrease at decreased perfusion pressure. Due to the scarcity of data, no statistical analysis could be made to differentiate between the effects of the short-term and the prolonged moderate reduction of perfusion pressure It might be argued that a PS decrease would have been demonstrated with statistical significance in the present study if all data had represented a prolonged period of hypotension. Judging from the few available measurements (see Fig. 7 below left) a tendency may be present to support this reasoning but a more extensive study would be necessary to justify a more conclusive comment.

Summary

The present data indicate a reduction of the exchange of small molecules in the dog's skeletal muscle during regional arterial hypotension (40 – 60 mm Hg). Compared to control values, the blood flow was reduced to 33 % and the PS product for ^{51}Cr–EDTA was reduced to 57 %. The change of ^{125}I-antipyrine extraction was insignificant. Data were too few to permit any statistical analysis to compare the effects of short-term and prolonged regional arterial hypotension.

BLOOD TISSUE EXCHANGE DURING HEMORRHAGIC HYPOTENSION

In two previous preliminary reports data were presented from studies on blood tissue exchange of small molecules in hemorrhagic shock by the indicator dilution method (Dahlberg and Lewis 1973 1976) In one of these studies (1976), a local clearance method was simultaneously employed. The results from the two reports are here analysed as a single group of data and with the same statistical approach as was used elsewhere in this monograph.

Experimental procedure

Hemorrhagic hypotension was produced by a rapid reduction of the blood volume decreasing the arterial blood pressure to about 50 mm Hg. By additional bleedings blood *pressure was maintained between 40 and 60 mm Hg.*

Blood tissue exchange was studied by the indicator dilution method and, in some experiments, simultaneously by the local clearance method. For the former method, different combinations of indicators were used in addition to ^{59}Fe-siderophilin which acts as an indiffusible vascular marker (see Table III). In 12 experiments ^{51}Cr–EDTA was paired with ^{125}I-antipyrine in seven, iodide ion was used instead of ^{51}Cr–EDTA (^{131}I, 5 experiments) or ^{125}I-antipyrine (^{125}I 2 experiments). In two experiments ^{125}I–RIHSA was used instead of ^{125}I-antipyrine (but together with ^{59}Fe-siderophilin).

Number of experiments	^{51}Cr–EDTA	^{131}I	^{125}I	^{125}I–RIHSA	^{125}I–antipyrine
5		*			*
2	*		*		
2	*			*	
12	*				*

Table III. Combinations of indicators used in addition to vascular marker for the indicator dilution method in experiments on hemorrhagic hypotension.

In the local clearance method the transport of ^{51}Cr–EDTA was studied by external detection following the intramuscular injection of this isotope mixed with ^{133}Xe. The ratio of their clearance values gives ^{51}Cr–EDTA extraction if ^{133}Xe clearance equals blood flow The technique was employed in ten experiments. For each set of measurements, tissue-to-blood transport was measured twice before and twice after two measurements of blood to-tissue transport. Measurements were carried out, apart from control state, at two four and six hours after the onset of the bleeding.

Results

Values for iodide ion (^{131}I or ^{125}I), ^{51}Cr–EDTA and ^{125}I-antipyrine from measurements by the indicator dilution method are presented in Table IV Mean values and the limits for the mean plus and minus one standard deviation are given for measurements before and after hemorrhage

	Control			2 hrs hemorrhage			4 hrs hemorrhage			6 hrs hemorrhage		
	N	Mean	Mean ± SD	N	Mean	Mean ± SD	N	Mean	Mean SD	N	Mean	Mean SD
Iodide ion (^{131}I, ^{125}I)												
Blood flow	7	3.9	2.6–5.8	7	1.5	1.1–2.1	5	1.4	0.8–2.2			
Extraction	7	0.36	0.29–0.46	7	0.48	0.38–0.60	5	0.52	0.43–0.63			
PS	7	1.77	1.11–2.82	7	0.99	0.57–1.73	5	1.01	0.77–1.32			
^{51}Cr–EDTA												
Blood flow	16	5.1	3.0–8.8	14	1.8	0.8–3.7	12	1.8	0.7–4.2	7	1.3	0.6–2.8
Blood flow LCM	9	7.3	4.6–12.0	9	4.3	2.6–6.9						
Extraction	16	0.15	0.07–0.33	13	0.23	0.12–0.43	12	0.22	0.15–0.35	5	0.24	0.16–0.36
PS	16	0.85	0.31–2.31	13	0.52	0.17–1.58	12	0.47	0.16–1.35	5	0.35	0.18–0.64
PS, LCM	9	0.94	0.73–1.23	9	0.49	0.34–0.71						
^{125}I-antipyrine												
Extraction	17	0.79	0.71–0.87	13	0.87	0.83–0.91	11	0.88	0.84–0.92	4	0.92	0.90–0.94

Table IV Mean blood flow extraction and permeability surface area values before and after hemorrhage. LCM indicates local clearance method.
Extraction values for ^{51}Cr–EDTA are only given for the indicator dilution method.

The effect of hemorrhage is presented in Table V. Any change for the lipid-insoluble indicators is given as the mean effect and a 99 % confidence interval. The results obtain

	Duration of hemorrhagic hypotension					
	2 hours		4 hours		6 hours	
	Effect %	Conf limits %	Effect %	Conf limits %	Effect %	Conf limits %
Iodide ion (^{131}I, ^{125}I)						
Blood flow	39	(21–72)	35	(11–110)		
Extraction	130	(87–176)	155	(71–342)		
PS	56	(26–118)	64	(35–116)		
Observed/expected extraction	70	(43–115)	78	(56–105)		
^{51}Cr–EDTA						
Blood flow	34	(22–54)	31	(18–54)	25	(12–52)
Blood flow LCM	58	(49–69)				
Extraction	137	(97–192)	201	(125–325)	164	(55–485)
PS	50	(25–97)	99	(27–131)	54	(10–279)
PS, LCM	51	(38–69)				
Observed/expected extraction	58	(35–95)	70	(41–120)	54	(17–171)
^{125}I-antipyrine						
Extraction (absolute difference)	0.06	0.01–0.11	0.08	0.02–0.14	0.09	0.01–0.19

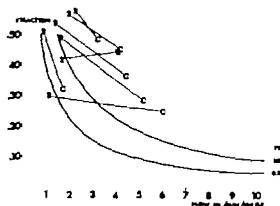
Table V. Change of extraction, blood flow and permeability surface area following hemorrhage. LCM indicates local clearance method.
The change in the extraction of ^{51}Cr–EDTA is only given for the indicator dilution method.

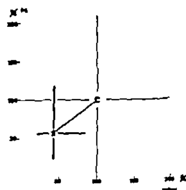
ed by the indicator dilution method are also presented in Figs. 8 and 9. Fig. 8 shows, to the left, the individual changes of iodide ion extraction and blood flow for each animal against a background of theoretical PS lines, after two and four hours of hemorrhagic hypotension. Fig. 9 shows the corresponding data for ^{51}Cr–EDTA after two, four and six hours of hemorrhagic hypotension. In both figures, to the right, the changes in blood flow and PS (in per cent of control) are presented in accordance with Table V. The largest PS reduction, 50 % for ^{51}Cr–EDTA after a two hour period of hemorrhagic hypotension, is statistically significant.

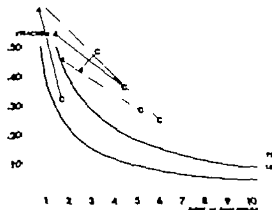
In the two experiments where ^{125}I–RIHSA was studied, this substance was found to behave in the same manner as ^{59}Fe-siderophilin.

^{125}I-antipyrine extraction was found to increase slightly after bleeding. The increase, however, was not statistically significant.

The results obtained by the local clearance method are also included in Tables IV and V. Fig. 10 shows *all* values (left panel) for Cr–EDTA extraction and blood flow (Xenon clearance) and also (right panel) the changes in blood flow and PS after *two* hours (in accordance with Table V). The PS reduction after two hours of hemorrhagic hypotension, to 51 %, is statistically significant.







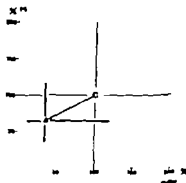
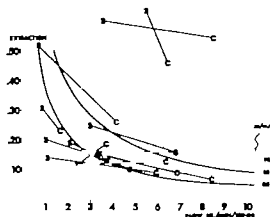
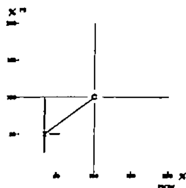
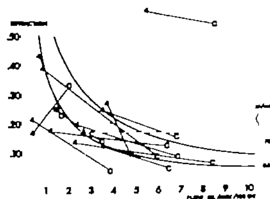


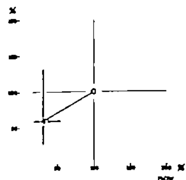
Fig. 8 Single-injection multiple-indicator dilution method. Extraction changes for *iodide ion* induced by *hemorrhagic hypotension* (left in figure). Data at two (2) hours (bove) and at four (4) hours (below) after the onset of rapid bleeding. Perfusion pressure approx. 50 mm Hg.

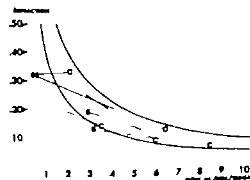
Changes in blood flow and PS (paired comparisons) in per cent (right). Mean values of PS and Q with 99 % confidence limits are given.











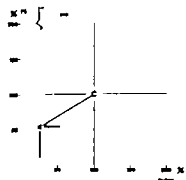
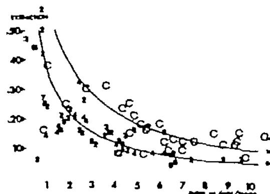


Fig. 9 Single-injection multiple-indicator dilution method. Changes in extraction and PS for Cr–EDTA at two (2) four (4) and six (6) hours during *hemorrhagic hypotension* (perfusion pressure approx. 50 mm Hg).

The decrease in PS is statistically significant (both confidence limits less than 100 %) at two (2) hours after the onset of bleeding. The number of surviving animals is gradually reduced during the shock period.



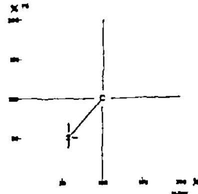


Fig. 10. Local clearance method. Extraction/flow diagram to the left, changes in PS and flow (in per cent) to the right.

Changes in extraction of Cr–EDTA during *hemorrhagic hypotension* compiled data from ten experiments where the method has been employed simultaneously with the indicator dilution method. Measurements were made in the control state (C) and at various times during the shock period, two (2) – seven (7) hours after the onset of bleeding.

The changes in PS and blood flow in per cent (right) indicate that the decrease in the values for these parameters (paired comparisons) is statistically significant after two (2) hours of hypotension (cf. data for Cr–EDTA obtained by indicator dilution method Fig. 9).

Comments on results

The extraction values of iodide were found to be slightly higher than those of Cr–EDTA. This was expected to occur as a consequence of the different molecular dimensions of the two substances. During the course of the hemorrhagic shock there was an increasing number of animal deaths and the scattering of PS values was therefore seen to increase.

Summary

During hemorrhagic shock, the blood tissue exchange in the dog's skeletal muscle was found to decrease. The transport of small lipid insoluble molecules between the blood and the interstitial fluid diminished markedly: a 50 % reduction of the capillary diffusion capacity (PS) was estimated both by the indicator dilution method and by the local clearance method.

BLOOD FLOW AND BLOOD TISSUE EXCHANGE IN TRAUMATIZED SKELETAL MUSCLE

In the present chapter data are presented which were obtained in studying the blood tissue exchange in injured skeletal muscle by the indicator dilution method. The hind leg musculature of the dog was traumatized by a series of contusions over a wide area. It was attempted to find out

1) If injury produces, consistently an initial increase in blood flow
2) How the PS response is related to the response in blood flow

Experimental procedure

The anaesthetized dogs (10) were ventilated with intermittent positive pressure ventilation. After control measurements (first and second isotope injection = C) injury to the dog's left hind leg was inflicted by delivering 200 heavy strokes from a blunt wooden instrument. Following this, one injection (three isotopes in plasma) was made as soon as possible within the first few minutes (D), a second 10 minutes (E), a third 20 minutes (F) and a fourth 60 minutes (G) after the contusion.

Results

All animals survived the whole period of observation. In one experiment, all extraction values, both during the control state and after the trauma were negative probably as a result of a technical error In this animal, there was a temporary blood flow increase following the trauma (not illustrated). Fig 11 (upper panel) shows the blood flow rates for each experiment. The double vertical line indicates trauma. In four of these experiments a *temporary* blood flow *increase* was seen to follow the injury giving, in total a flow increase early after trauma in five of the ten dogs. In five experiments a blood flow *decrease* followed, only in two of these the blood flow tended to return towards control level. The *dogs that responded to injury with a blood flow increase displayed* a blood *pressure re*duction that was less marked than the one shown initially by the others. In the two dogs that tended to improve by increased blood flow after an initial decrease, a parallel blood pressure restoration was observed. The level of blood flow or arterial pressure at control was not related to the blood flow response to the injury

Fig. 11 (lower panel) also shows the PS response to injury in each experiment. A PS increase was only seen in one experiment, where a single control value represented a relatively low PS value at low blood flow (III:6). Three dogs showed a prolonged PS decrease despite initial increase in blood flow If blood flow decreased, PS always decreased. Thus, PS *increase* was rarely seen, whereas *blood flow increase was seen in half of the experiments.*

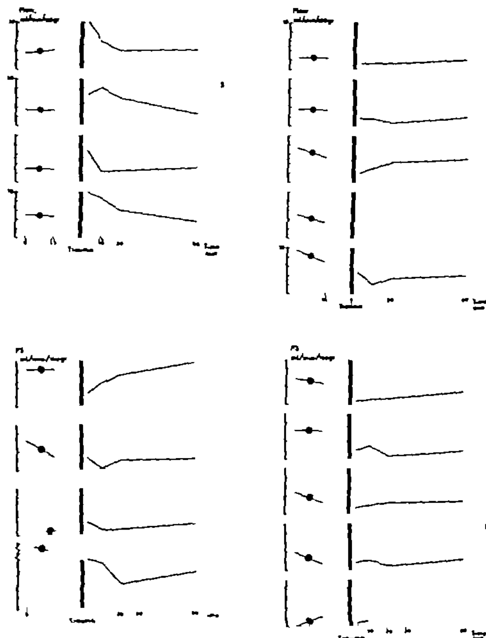


Fig. 11 Response in blood flow and PS (Cr–EDTA) to *injury* (single-injection multiple-indicator dilution method)

The blood flow (upper graphs) was measured immediately after the trauma and also after 10, 20 and 60 minutes. The mean control value is indicated by a closed circle. In four experiments there was an increase in blood flow (left) maintained for varying period of time. In five experiments, the blood flow decreased after injury (right).

The response in PS is seen in the lower graphs. With one exception (experiment 6) PS was reduced after injury

Fig. 12 shows individual extraction values for ^{51}Cr–EDTA at the actual blood flows *before (C) immediately following (D) and 10, 20 and 60 minutes after trauma* (E, F and G). There is a separate diagram for each dog.

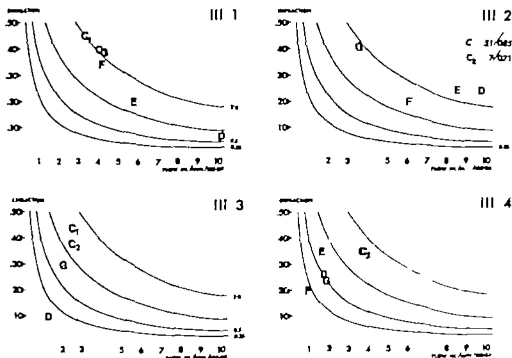


Fig. 12a. Response to *injury*. Extraction/flow values (Cr–EDTA) obtained by the single-injection multiple-indicator dilution method. Control values are indicated by C_1 and C_2. The values obtained after injury are indicated by D, E, F and G (<5, 10, 20 and 60 minutes). The curves are drawn f constant values f PS product shown in the right margin. In one of the experiments (III 2) indicating temporary increase in blood flow after injury the control values for extraction of Cr EDTA are exceptionally high.

If the change of extraction values was analysed with respect to the expected change at constant PS a statistical analysis revealed an effect in accordance with Table VI.

Observed/expected extraction

D	E	F	G
57 %	57 %	63 %	80 %
(22–144) %	(19–171) %	(30–133) %	(45–140) %

Table VI. Change of the value for observed/expected extraction for ^{51}Cr–EDTA induced by injury

D–G denote time after trauma: immediately 10 20 and 60 minutes. The response was most pronounced initially in the period following injury. As stated earlier the response in PS should be similar (cf Fig. 13). The changes, however were not uniform or statistically significant.

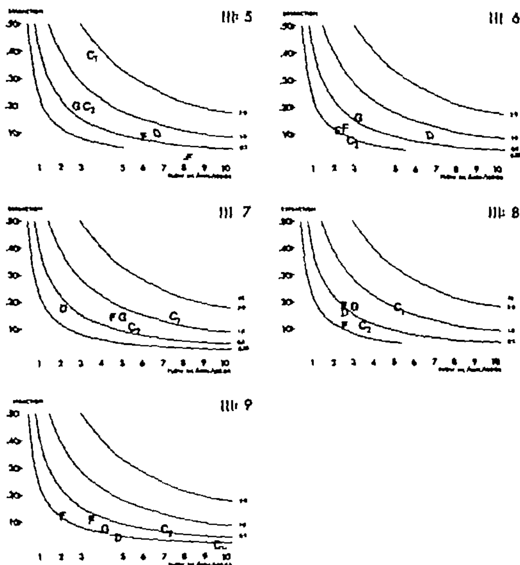


Fig. 12b. Response to injury. Extraction/flow values (Cr–EDTA). In one experiment (Ill 6) only one value for Cr–EDTA extraction was obtained, representing a PS value for the control state that is remarkedly low. Injury is seen to induce, in this experiment, an initial increase in PS.

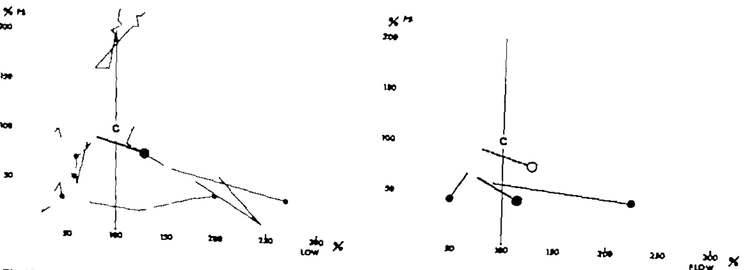


Fig. 13. Response to *injury.* In the left graph, response in $PS_{Cr\text{-}EDTA}$ and blood flow presented for each experiment. Changes are given in per cent of control values (C). Small filled circles represent first values obtained immediately after injury and then lines connect subsequent values after 10, 20 and *60 minutes. In all experiments except one,* PS is decreased in comparison with control. The larger filled circle with the thick line indicates the mean effect of injury in the nine experiments and represents the values *obtained immediately after injury (filled circle)* and after *60* minutes (end point of line). If the only experiment with an increase in PS is excluded this symbol is displaced towards lower values for PS as illustrated *in the right graph.* The smaller filled circles (right graph) represent the mean effects of injury if the same data are divided according to initial response in blood flow

In Fig. 13 (left graph) the response in PS and Q is presented for each experiment. The changes are given in per cent of the control values (C). Except in one experiment, PS was markedly reduced and the response in PS was seen to be unrelated to the change in blood flow In the right part of the figure the mean effects are presented with respect to the different early response in blood flow i. e. flow increase or decrease after trauma. The decrease in PS was present within a few minutes after the period of traumatization. During the following hour there was a slight increase in PS towards control level and the blood flow rate was returning towards pre-trauma values.

^{125}I–antipyrine extraction was unaffected by the injury The mean, statistically in significant, difference was 0.014 (S.E. 0.026).

Comments on results

The response in PS for Cr–EDTA to injury seems to be independent of the change in total blood flow This may indicate that the mechanisms which control the distribution of capillary blood flow are differently influenced by injury in comparison with those that govern the total blood flow

Summary

Blood flow and blood tissue exchange of ^{51}Cr–EDTA and ^{125}I-antipyrine were studied in the dog's skeletal muscle before and after local injury Following injury total blood flow immediately increased in one half of the experiments. In the other animals total blood flow decreased. PS was nearly always seen to decline after injury even when total blood flow increased.

CHAPTER 8

BLOOD FLOW AND BLOOD TISSUE EXCHANGE DURING NORMOVOLEMIC HEMODILUTION

In this chapter data are presented which were obtained in studying blood tissue exchange of ^{51}Cr–EDTA by the indicator dilution method at various levels of normovolemic hemodilution. In some of the experiments the local clearance method was simultaneously used. The results have been analysed in accordance with the method used elsewhere in this monograph. The statistical analysis is different in comparison with a previous, preliminary report on some of the data (Dahlberg and Lewis 1975)

Experimental procedure

The animals were initially splenectomized. Control measurements were made before the hemodilution started. For hemodilution, equal amounts of whole blood were exchanged for dextran 70 (Macrodex®, six per cent dextran in saline). Measurements were repeated at hematocrit levels of 25–30 % and 10–12 %. At each hematocrit level two measurements of tissue-to-blood extraction were made before and after a pair of measurements of blood to-tissue extraction. $^{59}FeCl_3$ ^{51}Cr–EDTA and ^{125}I-antipyrine were used as indicators for the indicator dilution method, ^{51}Cr–EDTA and ^{133}Xenon for the local clearance method.

Results

Fifteen experiments were carried out. The indicator dilution method was used in all of these. Simultaneously the local clearance method was used in six experiments (in one of which the blood-to-tissue values had to be discarded because of a technical error). Due to hematocrit changes during hemodilution all flow rates are given as plasma flows and PS (Cr–EDTA) values are given in ml plasma/min/100 g tissue.

The mean values for flow ^{51}Cr–EDTA extraction and PS for Cr–EDTA at control and after hemodilution are presented in Table VII.

The effect of hemodilution is presented in Table VIII. In Fig. 14 individual plasma flow changes and ^{51}Cr–EDTA extraction changes (indicator dilution method) are shown at the two stages of hemodilution (left panels). Open circles represent values at hematocrit 25 – 30 % filled circles values at hematocrit 10 – 12 %. The changes in plasma flow and PS are also seen in Fig. 14 (right panels) in accordance with Table VII. A progressive increase in flow and PS is seen to follow the hemodilution.

Fig. 15 (left panel) shows the compiled data for tissue-to-blood ^{51}Cr–EDTA extraction (local clearance method) at control (C) hematocrit 25–30 % (open circles) and hematocrit 10–12 % (filled circles). From the figure it appears that PS changes mea-

	Control			Level 1			Level 2		
	N	Mean	Mean ± SD	N	Mean	Mean ± SD	N	Mean	Mean ± SD
^{51}Cr EDTA									
Plasma flow	14	2.2	1.6–3 1	14	3 4	2.4–4 8	13	4.5	3 0–6 7
Plasma flow LCM	6	3.8	2.0–7 0	6	5.9	2 6–13 7	6	7.5	3.8–15 1
Extraction	14	0.23	0 10–0.52	14	0.25	0 13–0 48	13	0.23	0 14–0.38
PS	14	0.62	0.23–1 62	14	1.02	0.52–2.00	13	1.31	0 76–2.28
PS LCM	6	0.65	0.50–0 85	6	0.74	0.62–0 88	6	0.57	0 43–0 76
^{125}I-antipyrine									
Extraction	14	0.80	0 70–0.90	14	0.77	0 67–0.87	13	0.85	0.80–0.90

Table VII. Mean plasma flow extraction and permeability surface area before and after normovolemic hemodilution. LCM indicates local clearance method. Extraction values for ^{51}Cr EDTA are only given for the indicator dilution method.

	Level of hemodilution			
	Effect[1] %	Conf. limits %	Effect[2] %	Conf limits %
^{51}Cr–EDTA				
Plasma flow	153	(127–184)	215	(167–276)
Plasma flow LCM	156	(101–239)	198	(126–311)
Extraction	112	(82–156)	107	(64–177)
PS	165	(105–259)	222	(137–358)
PS LCM	113	(87–147)	88	(51–151)
Observed/expected extraction	165	(113–243)	?10	(119–370)
^{125}I-antipyrine				
Extraction (absolute difference)	–0 01	(–0 07–0 05)	0 07	(0 01–0.13)

Table VIII. Change f plasma flow extractio and permeability surface area following normovolemic hemodilution. LCM indicates local clearance method.

The change in the extraction of ^{51}C –EDTA is only given for the indicator dilution method.

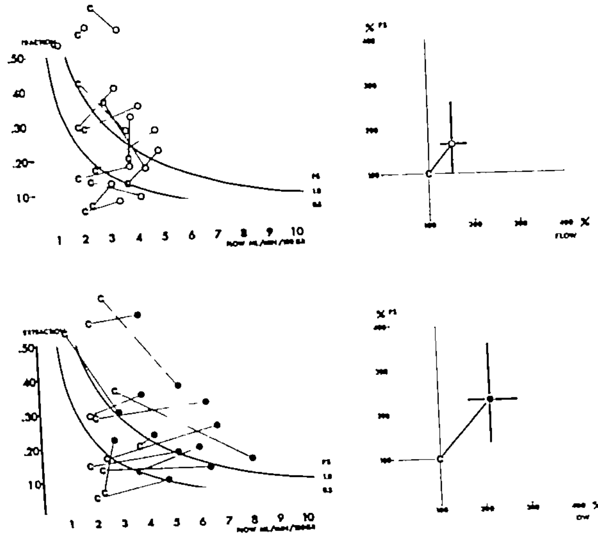


Fig. 14 Effects of *normovolemic hemodilution*. Single-injection multiple-indicator dilution method (14 experiments). Extraction (Cr–EDTA)/*plasma flow* diagrams to the left, changes in PS (Cr–EDTA) and *plasma flow* (in per cent) to the right.

The upper graphs represent the first level of hemodilution (hematocrit 25 – 30 %). Values obtained (indicated by open circles) in relation to control values (C).

The lower graphs represent the second level of hemodilution (hematocrit 10 – 12 %). Values obtained (indicated by filled circles) in relation to control values (C).

The changes in PS (Cr-EDTA) and plasma flow are statistically significant at the two levels of hemodilution. Note the progressive increase in both plasma flow and PS (Cr–EDTA).

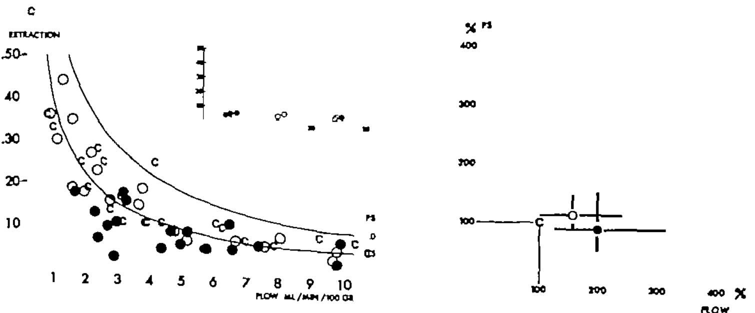


Fig. 15 Effects of normovolemic hemodilution. Local clearance method (used simultaneously with the indicato dilution method; 6 experiments).

T the left, extraction (Cr–EDTA)/*plasma flow* diagram for the compiled data. Open and filled circles represent values obtained at two levels of hemodilution (hematocrits 25 – 30 % and 10 – 12 %). The *plasma flow values (Xenon clearance)*, including those for the control state (C) are spread over a wide range (cf. Fig. 14 indicator dilution method).

T the right, the mean values for the changes in PS and plasma flow (paired comparisons with control). The 99 % confidence *limits indicate that the increase in plasma flow is statistically significant* at both hemodilution levels, whereas the changes in PS (Cr–EDTA) are negligible (in comparison with Fig. 14 indicator dilution method) and statistically insignificant. Not that the confidence limits for plasma flow are larger than those obtained by the indicator dilution method (Fig. 14).

by the local clearance method following hemodilution are small or none as compared to PS changes measured by the indicator dilution method. A statistical analysis supports this impression. Mean values are seen in Table VII the changes in Table VIII and in Fig. 15 (right panel).

The change in ^{125}I-antipyrine extraction following hemodilution was statistically insignificant.

Comments on results

Since the indicators are transported in the plasma, the PS (Cr EDTA) values are based on plasma flow rates rather than rates of whole blood flow During hemodilution with a varying content of plasma in the blood, the change of plasma flow is therefore more relevant than the change of blood flow when considering substances like ^{51}Cr–EDTA which are transported exclusively in plasma. PS expressed in this way is independent of hematocrit if measured under identical conditions, i. e. in a vasodilated vascular bed at high flows (Appelgren 1972b).

Summary

Capillary transport of ^{51}Cr–EDTA and ^{125}I-antipyrine was studied in the dog's skeletal muscle during graded, normovolemic hemodilution. The indicator dilution method was employed in all experiments, in some of the experiments the local clearance method was simultaneously used. The plasma flow and PS for ^{51}Cr–EDTA were found to increase with hemodilution when measured by the indicator dilution method. The results obtained by the local clearance method confirmed the increase in plasma flow but no change in PS was seen when measured with this method.

CHAPTER 9

INFLUENCE OF INCREASED VENOUS PRESSURE ON TOTAL BLOOD FLOW AND BLOOD TISSUE EXCHANGE IN THE DOG HIND LEG

In the previous chapters, data have been presented from studies dealing with the blood tissue exchange of small molecules in the musculature of the dog's hind leg. The data represent both the control state and several activated states such as regionally decreased perfusion pressure, hemorrhagic hypotension, blunt tissue trauma and hemodilution. It has been assumed, when the blood-to-tissue exchange was measured, that the total venous return in the iliac vein represents the total blood flow through the musculature and its capillaries.

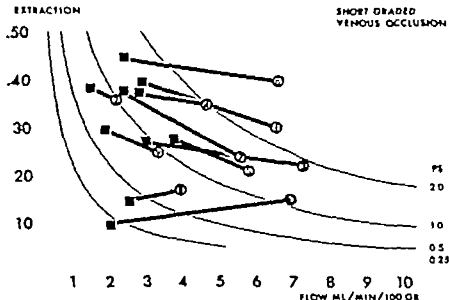
Although it is generally assumed that convection exchange is of minor importance in the blood-to-tissue exchange of molecules as small as Cr–EDTA, it was acknowledged that an increase of the venous pressure, if large, could possibly influence the transport of this indicator.

In order to study how an increased venous pressure affects the blood flow and the blood-to-tissue exchange, experiments were carried out where the regional venous blood pressure in the femoral vein was elevated for varying periods of time.

Experimental procedure

The preparation and analytical procedures have previously been described. Some experiments were made to study the effects of a repeated, short term increase of venous pressure. The venous pressure was then temporarily raised by elevating the free end of the T tube leading from the iliac vein, 13–14 cm above the level of the heart during the blood sampling period. In some experiments the venous pressure was elevated to the same extent (10 mm Hg) for longer periods by a partial occlusion of the iliac vein proximally to the T-tube. The T-tube was then linked to a saline manometer, disconnected only when the T-tube was used (in elevated position) for blood sampling. Care was taken not to inject the indicators until a constant venous blood flow could be observed following the venous pressure elevation. The venous pressure was not increased for more than 3 minutes with intervals of at least 15 minutes between repeated measurements. When venous pressure was raised continuously for several hours, measurements were made during the second, third and fourth hour after the onset of pressure elevation. No direct adjustments of arterial blood pressure were made during the course of experiments.

When the venous pressure was elevated, the blood flow through the iliac vein was regularly found to be markedly reduced but the perfusion pressure only slightly decreased in comparison with control. This was the case regardless if venous pressure was elevated for a short or long period of time (Fig. 16).



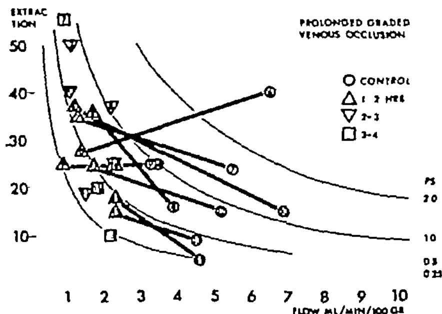


Fig. 16 E traction (Cr EDTA) and blood flow values obtained after graded occlusion of the iliac vein in the non-isolated hind limb of the dog. Blood flow was measured by timing the venous return in the iliac vein The *increase in venous pressure* was 10 mm Hg. The numbers within the open symbols indicate the individual experiment The open circles are the control values before the elevation of venous pressure

The upper graph represent measurements made during periods of *short-term* elev tion of venou pressure

Values were lso obtained (lower graph) during periods of *prolonged* elevation of venous pressure

To clarify this finding, both arterial and venous blood flows were measured simultaneously both at normal and at increased venous pressure in a separate series of experiments. Arterial blood flow was measured by an electromagnetic flow-meter (femoral artery) and venous blood flow was measured by timing venous return. Three experiments were carried out for this purpose with the same basic surgical preparation as was used in the others. In one of these experiments, ligatures were initially inserted through the musculature as far proximal in the thigh as possible. When tightened around the muscles the ligatures excluded the main femoral vessels and the sciatic nerve so as to obstruct collateral blood pathways.

$^{59}FeCl_3$, ^{51}Cr–EDTA and ^{125}I-antipyrine were used as indicators.

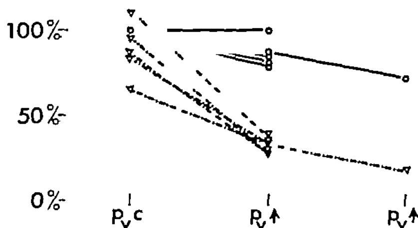
Nineteen experiments were carried out. In five experiments measurements were made during four repeated periods of the short term increase of venous pressure. From some of the other fourteen experiments, additional data were obtained soon after the venous pressure was elevated (five exps.) subsequent measurements in all the fourteen experiments were made to study the effect of a prolonged regional venous hypertension.

Results

Fig. 17 shows results from the comparative measurements of arterial and venous blood flows. In the upper panel (A), blood flows are given in per cent of the arterial blood flow rate at control level of venous pressure (p_vc). Five values were obtained after a short term increase of venous pressure (3 min.) and one value after a longer increase (30 min.). Of interest is the good agreement between arterial and venous blood flow during the control period. As venous pressure was raised (10 mm Hg) the arterial blood flow decreased to 90 % and venous blood flow to 1/3 of its control level. The perfusion pressure was reduced by 10 mm Hg from a control level of 100 – 120 mm Hg. After 30 minutes the blood flow had decreased further. In the lower panel of Fig. 17 (B) the blood flows are shown after ligation of the musculature (collateral blood flow). As venous pressure was raised, venous blood flow decreased at a much smaller degree and to the same extent as arterial blood flow.

In Fig. 16 the upper panel shows ^{51}Cr–EDTA extraction values following a short term pressure increase the lower panel ^{51}Cr–EDTA extraction values following a prolonged increase of venous pressure. Analysis of the venous blood flow rate shows a reduction to 54 % following the short-term increase in venous pressure. At continuous increase of venous pressure venous blood flow is reduced to 40 % after 1–2 hours and to 33 % after longer periods, both in relation to control flow. This is in fairly good agreement with the results shown in Fig. 17 (upper panel). The PS change was not statistically significant in any of the periods with increased venous pressure.

Fig. 18 shows the data from Fig. 16 following recalculation of venous blood flow values in accordance with the findings from the separate experiments (Fig. 17). With these cor



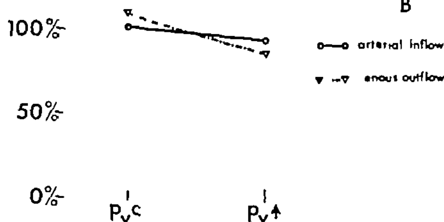
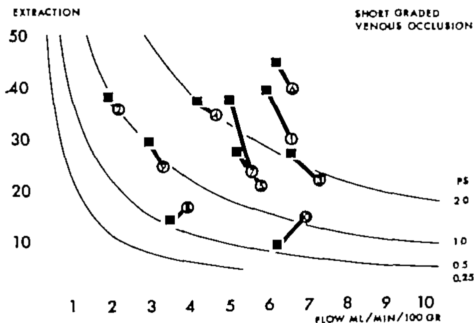


Fig. 17 Comparative measurements of arterial and venous blood flows at normal ($p_v c$) and elevated (p_v, arrow) venous pressure. Values obtained in the non-isolated hind limb preparation (graph A) and in the isolated preparation (graph B). Open circles indicate arterial blood flow measured by an electromagnetic flow probe on the femoral artery triangles indicate blood flow measured by timing venous return from the iliac vein.

The arterial inflows before elevation of venous pressure ($p_v v$) were set to 100 %. The arterial blood flow values with increased venous pressure (p_v, arrow) and all values for venous blood flow were expressed in per cent of the initial values for arterial blood flow

Most measurements were made following control, during short-term elevation of venous pressure; the single values near the right margin of the upper graph represent values obtained after a 30 minute period of increased venous pressure



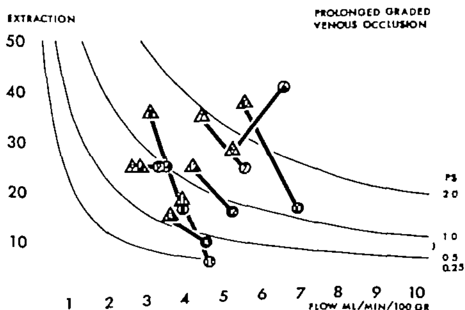


Fig. 18 Cf text to Fig 16. The values for blood flow in the two diagrams have been recalculated according to the relation found between the arterial inflow and the registered venous outflow as illustrated in Fig 17

rected" values for blood flow the PS change, induced by the venous pressure decrease, remained practically unchanged both following short-term and prolonged pressure elevation.

^{125}I-antipyrine extraction was not affected by any duration of venous pressure elevation, except after 1 – 2 hours of prolonged elevation when a small decrease was observed, mean difference 0 003 (SE 0 007)

Comments on results

In the present study the total blood flow of the dog's hind leg was measured by collecting the total venous return in the iliac vein. At control, this venous blood flow was found to be of the same magnitude as the arterial blood flow When the venous pressure was raised, however the venous blood flow decreased markedly By measurement of the arterial blood flow it was found that the venous flow reduction equalled the amount of collateral venous return, i. e. the difference between the blood flow measured in the femoral artery and in the iliac vein. In contrast, the arterial blood flow through the hind leg decreased by only 10 % when the venous blood pressure was raised (10 mm Hg). This blood flow reduction is consistent with the moderate reduction of perfusion pressure The additional blood flow reduction, seen after a longer period of venous pressure elevation, is probably due to the gradual perfusion decline observed throughout the experiment.

The observed ^{51}Cr–EDTA extraction values after venous pressure increase did not statistically differ from theoretically expected values at an unchanged PS. This was the case at any duration of venous pressure increase and regardless if the venous blood flow data were corrected or not with respect to the comparative calculations. It has been proposed that blood samples taken from the iliac vein are representative of blood from the entire capillary bed not only at control but also at increased venous pressure, when an increased collateral venous return is present.

Summary

The blood to-tissue transport was studied in the innervated dog hind limb at moderately increased venous pressure (10 mm Hg). Multiple-indicator dilution curves for $^{59}FeCl_3$, ^{51}Cr–EDTA and ^{125}I-antipyrine were obtained from the outflow through the iliac vein. The venous outflow was also used for measurement of the total blood flow The total venous return in the iliac vein at normal venous pressure was found to be representative for the total blood flow in the hind leg.

It was found that the external pressure exerted on the iliac vein to raise the venous pressure caused a considerable diversion of the blood flow from the hind leg into collaterals The transport of ^{51}Cr–EDTA and ^{125}I-antipyrine from blood to tissue was not changed by the elevation of the venous pressure

CHAPTER 10

GENERAL DISCUSSION

A. Methodological considerations

Some of the difficulties concerning the theory behind the multiple indicator dilution method and the local clearance method are referred to model assumptions about capillary wall structure and hemodynamics and are made very evident when a mathematical relationship between such parameters as blood flow Q extraction, E and permeability surface area product, PS is used for studies on the vascular bed of a whole organ. The difficulties are even more conspicuous when low flow states are to be studied and compared with normal flow states. Some factors concerning these problems will therefore be discussed in more detail.

1 Blood flow Q

Throughout the present studies, the blood flow through the musculature of the dog's hind leg was estimated by measuring the total venous outflow in the iliac vein. In the control state, there are no obvious reasons to doubt that the total inflow of blood should be well represented by this measurement. This was confirmed in the present study when arterial inflow and venous outflow were simultaneously measured during the control state (see Chapter 9).

In previous studies with a surgical preparation similar to the present, venous [illegible] measurements were found to agree well with the arterial inflow measured with an [illegible] magnetic flowmeter particularly during the control state (Sandegård 1974). [illegible] trauma, however a significant increase of blood flow was noticeable for a [illegible] of time (15 minutes) when measured from venous outflow than it was when [illegible] from arterial inflow (30 minutes). This may indicate that the inflow [illegible] become inaccurate or that venous outflow measurements did not [illegible] the total blood flow through the vascular bed. The second explanation [illegible] development of a collateral venous outflow leading to a smaller increment [illegible] venous outflow as compared to arterial inflow for an initial period [illegible] ter trauma.

In the present study the presence of recruitable outflow vessels [illegible] clearly demonstrated when the iliac vein was compressed the [illegible] sel only remained unchanged in relation to arterial inflow when [illegible] by mass ligatures.

In conditions with a decreased perfusion pressure and a [illegible] as arterial hypotension and hemorrhagic hypotension, there [illegible] similar diversion of venous outflow affecting the measurement [illegible]

However in a hyperaemic state such as normovolemic hemodilution the presence of recruitable collaterals may possibly interfere with the measurement of total blood flow from venous return. The correction for an underestimation of flow after normovolemic hemodilution would give an even higher flow with an unchanged or increased PS. Such a correction would, if anything, enforce the evidence leading to the conclusion of an increased PS and increased flow after normovolemic hemodilution.

2. Extractions E

a) I-antipyrine a lipid-soluble molecule. The transport through the capillary wall of lipid-soluble molecules is not only confined to the differently sized pores that allow water and lipid-insoluble (water-soluble) molecules to cross the endothelial cell layer Data from studies by Renkin (1952, 1953) indicated that lipid soluble molecules also diffuse rapidly *through the cell membrane material. The* entire surface area of the capillary wall may thus be utilized for blood tissue exchange of a molecule like antipyrine which is lipid-soluble The extraction of the molecule will be independent of blood flow within a wide flow range and therefore its clearance (Q x E) will vary only with varying flow The actual value of fractional extraction will be close to unity in a vasodilated tissue but lower in the resting state where non-uniformity of capillary blood flow distribution will act as a physiological shunt (Chinard et al. 1955 Renkin 1955 1959 Landis and Pappenheimer 1963).

In the present study the extraction of I-antipyrine was 0.79 (mean control value) and it remained independent of flow in the various activated states.

b) Cr–EDTA a lipid-insoluble (water-soluble) molecule. The curves that were obtained for the fractional extraction of Cr–EDTA showed in their initial parts, variable and usually high extraction values soon after injection of isotopes (see Fig. 4). High values were interpreted as being due to interlaminar diffusion, giving rise to different dispersion intravascularly for the participating diffusible (Cr–EDTA or iodide) and indiffusible (Fe-siderophilin) tracer the so-called Taylor-effect (Taylor 1954). A plateau of constant extraction values, appearing in several samples around peak time for the curve of non-exchanging tracer was taken as evidence that the Taylor-effect had subsided and that back diffusion was minimal Extraction values were estimated only when a series of extraction values were found to form a plateau.

Low initial values for extraction rising towards the plateau have previously been seen in non-vasodilated tissue (Rose and Goresky 1976).

In several earlier studies, the weighted means of the separate extraction have been calculated in order to lessen the effects of extraction variation with time (Crone 1961). Other methods have been proposed by Martin de Julián and Yudilevich (1964) and by Bassingthwaighte et al (1970)

3 PS PS/Q quotient of Cr–EDTA or iodide and back diffusion

In resting muscle one fourth to one half of the total capillary face is perfused (Pappenheimer 1953 Kjellmer 1965 Renkin et al. 1966 Mellander and Johansson 1968). It is often assumed that the blood flow is uniformly distributed in the open capillaries with respect to their individual surface, i.e. the capillaries have identical S/Q ratios, permeability and extraction values. This would give PS values calculated in accordance with the equation $PS = -Q \ln(1 - E)$.

Total clearance of a substance may be predominantly achieved by extraction in relatively well-perfused capillaries which would, therefore only represent a part of the capillary population. It should be kept in mind, however that the clearance in capillaries receiving a larger fraction of blood cannot compensate for the decreased clearance in less effective capillaries (Renkin 1959 Renkin and Rosell 1962, Renkin 1961 1964 Renkin et al. 1966) If a number of capillaries were to be obstructed, PS cannot remain unchanged unless the degree of non-uniformity among remaining perfused capillaries is decreased or more capillaries recruited.

Non-uniformity of S/Q ratios and, hence capillary blood flow distribution leads to a lower value of calculated PS product during control and may if enhanced during activated states, explain additional apparent reductions of PS.

Changes of calculated PS are known to be induced by changes of blood flow This is the case both in resting and vasodilated skeletal muscle (e.g. Renkin 1959 Trap-Jensen and Lassen 1970 Appelgren 1972, Paaske 1977 Rippe et al. 1978). The change in PS with flow *particularly prominent at lower flow levels* represents a functional rather than a geometrical alteration of the capillary surface.

In studies on blood tissue exchange, vasodilatation is often induced in order to reduce the effects of non-uniformity of capillary blood flow In the present kind of experiments, such a procedure would probably induce too deleterious effects on central hemodynamics in the animal and was for this reason not practised.

It is, however evident that experimentally observed changes in PS have to be evaluated in relation to any existing changes in flow In a low flow state, factors related to the duration and magnitude of prevailing perfusion pressure may also have a specific bearing on PS (Appelgren 1972 cf Järhult and Mellander 1974). At very low flow levels, PS measurements may very well become erroneous as a consequence of back diffusion, but also due to high PS/Q quotients, indicating flow-limitation, thus is to be expected when the value of PS/Q is exceeding 0.5 (Goresky et al 1970, Bassingthwaighte 1974 Guller et al. 1975 Rippe 1978). In the present study the value of the PS/Q quotient was usually well below this figure

Even if the total blood flow through a musculature were to be accurately measured it is not necessarily representing the total blood flow through the open capillaries (Renkin 1959 Renkin et al. 1966 Michel 1971) The existence in the resting muscle or the open-

ing in activated states of short arterio-venous interconnections (shunts) would produce very low PS/Q quotients which weighted for their flow in the calculation of total PS/Q would result in low PS values for the entire capillary bed.

4 Blood to tissue vs tissue to blood transport

In some series of the present experiments (hemorrhage and hemodilution) two different methods were employed to study the blood tissue exchange. An attempt has been made to present the data from these methods in a conformed manner in extraction(Cr–EDTA)/flow diagrams.

It has occasionally been argued that indicators delivered to the tissue via the arterial blood stream would always stand a better chance to reach well-functioning capillaries than indicators which are directly deposited in the interstitial fluid. However any change in the number or in the functional capacity of those capillaries (per unit tissue weight or volume) which participate in diffusion transport, would affect the over-all diffusion capacity of the capillary bed regardless of the direction of the diffusional transfer and it is only when the tissue produces a considerable additional extravascular diffusion barrier for substances like ^{51}Cr–EDTA that PS(tissue to blood) will be less than PS(blood to tissue)

In the situations where data were obtained simultaneously from the indicator dilution technique and the local clearance method, these data were in agreement during hemorrhagic hypotension and diverse during normovolemic hemodilution. Possible explanations for this finding will be discussed below

B The control state

In the present investigation, blood tissue exchange was studied in a resting skeletal musculature The mean blood flow was 4.6 ml/min/100 g, the mean PS for Cr–EDTA 1 15 ml/min/100 g The PS values were not significantly related to the flow values, the two parameters should be imagined as highly independent of one another each being related to the vascular tone prevailing in a different serial segment of the blood vessels. The large scattering of Q and PS values may to a great extent, depend on the varying degree of influence exerted on the blood vessel musculature by the wide range of mechanisms that is normally operative to govern blood flow and blood tissue exchange (Mellander and Johansson 1968)

The distribution of control PS values for Cr–EDTA is not a Gaussian one This has also been observed in other studies on diffusion solute exchange across the capillary wall (e g. Crone et al 1978, cf. Crone and Christensen 1979). The skewed curve of distribution (see Fig. 6) is reminiscent of the one observed for values of the filtration coefficient (e g. Pappenheimer et al. 1951 Michel et al. 1974) and indicative of a variation in the permea

bility properties between individual capillaries or capillary beds. The possible causes for the variation in the values of PS (and filtration coefficient) are not, as yet, fully understood.

No significant correlations were found to exist between the values for Q and PS and various background variables which could possibly be related to the degree of circulatory disturbance at the time for control measurements.

Although much of the spread in magnitude of PS (and flow) changes is due to control differences, these differences, however are not necessarily produced by a varying severity of preparation trauma (see Chapter 4). Furthermore the mode of reaction in an activated state is often more important physiologically from a relative quantitative or qualitative than from an absolute quantitative point of view

The control Q and PS values are of some interest in relation to changes induced in different activated states since the magnitude of the changes in paired comparisons could be expected to depend on the initial level of the control value (varying from preparation to preparation) This was clearly demonstrated by Renkin and Rosell (196?), at sympathetic chain stimulation the magnitude of the change in resistance was seen to depend on the chosen rate of blood flow and on the vascular resistance which was prevailing in the control state The change of PS was not influenced by variation of blood flow nor was it dependent on the simultaneously induced change in resistance Apart from concluding that the change in PS was not effected by the increase in vascular resistance and that the resistance change was related to the level of vascular tone in control the authors also observed that the magnitude of the individual change in PS depended heavily on the difference of PS in control.

C. The activated state

1 The activated state with a decreased blood flow

a. Regional arterial hypotension. In previous studies on the effects of regional arterial hypotension on the tissue-to-blood transport of low molecular size solutes, it was found that PS was more markedly and more consistently reduced (more than 50 %) following a one hour period of hypotension than within the first hour (Appelgren 1972). During the first hour there was an unsteady extraction rate as demonstrated by uneven slopes in the elimination rate curves (local clearance method). Several other studies on hypotension, including hemorrhagic shock, have also indicated that the time factor is of importance. Signs of autoregulation of the capillary pressure due to changes of the precapillary myogenic activity eventually disappear during prolonged arterial hypotension (Lewis and Mellander 196_, Jarhult and Mellander 1974) and fluid filtration from blood to tissue is seen to take place as a consequence of an increased post-capillary resistance (Mellander and Lewis 1963 Jarhult and Mellander 1974).

Järhult and Mellander (1974) also noted that the level to which perfusion pressure was reduced was decisive as to whether an increase of post-capillary resistance should arise during short term hypotension The critical pressure level appeared to be 40 mm Hg. Such an increase of post-capillary resistance is believed to be due to rheological factors, also causing an obstructed and non-uniform for diffusion transport less effective, capillary blood flow by venular stasis.

Thus, the extent of blood tissue exchange during hypotension may be expected to de pend to some extent, on the level of arterial pressure during short term hypotension and on the duration of the hypotension period during prolonged hypotension.

The flow disturbance caused by capillary obstruction should markedly affect PS which is known to be highly sensitive to changes in the number of perfused capillaries. This has been observed both in studies with the indicator dilution method and with the local clearance method (Appelgren 1972, Rippe et al. 1978). The capillary coefficient for filtration on the other hand, appears to increase during regional arterial hypotension (Cobbold et al 1963 Järhult and Mellander 1974) unless the hypotension is accompanied by massive capillary obstruction (Rippe et al 1978).

Partly in order to estimate the flow-dependence of PS a series of experiments was made where the arterial perfusion pressure was regionally reduced. As a result of the arterial hypotension PS for Cr–EDTA was seen to decrease. On the basis of the experiments, however, it cannot be decided to what extent the PS reduction is a consequence of the flow-dependence of PS per se or if any part of the reduction does in fact correlate with an absolute decrease in capillary surface area due to capillary obstruction by rheological factors, such as retardation of blood corpuscles.

b Hemorrhagic hypotension Changes which are induced by acute hypovolemia in the blood circulation of skeletal muscle seem to depend, among other things, both on the time factor and on the magnitude of the changes in perfusion pressure. At higher perfusion pressures, often initially present during hemorrhagic hypotension, the precapillary resistance is relatively more increased than the post-capillary As the hypotension period is prolonged, precapillary vessels tend to dilate under the influence of locally accumulated metabolites. The initial fluid absorption into the blood, enhanced by hyperosmolality will eventually decline and in very late stages of hemorrhagic shock both hydrostatic and probably also osmotic forces will initiate filtration (Mellander and Lewis 1963 Lundgren et al. 1964 Mellander and Öberg 1967 Järhult 1973 1975).

Acute hemorrhage has been found to give rise to a decrease in capillary diffusion capacity apparently due to a reduction in the number of capillaries which participate in blood tissue exchange Appelgren (1972) found that acute hemorrhagic hypotension, at perfusion pressures above 60 mm Hg, after one hour induced a 50 % reduction of PS Within the first hour the transport of iodide from the tissue to the blood was found to be unstable at reduced blood flow Very similar results were obtained when the blood flow to

the hind leg, at comparable perfusion pressure was reduced by occlusion of the aorta and the transport studied during a similar course of time These findings seem to be in line with observations indicating that rheological changes produced by hypotension r quire some time to develop and seem to be located towards the venous side in the lary bed (Järhult and Mellander 1974). Appelgren found an additional PS reduct pearing at perfusion pressures lower than 50 – 60 mm Hg. He postulated that th tional PS reduction was caused by an increased heterogeneity of capillary blood flow with reduction of the perfusion pressure rather than any further reduction in the number of perfused capillaries.

Signs of a widespread capillary obstruction following acute hemorrhage were also found by Baeckström et al. (1971) CFC was reduced to 40 – 50 % of control value while flow resistance was occasionally increased six fold.

Discussing the relationship between capillary blood flow and coefficients for capillary filtration and diffusion, Renkin (1969) emphasized that diffusion transport is more sensitive than filtration to changes in capillary blood flow To explain disproportionate changes of CFC and PS he argued that if flow distribution is controlled by a graded activity of terminal arterioles and precapillary sphincters, such a mechanism would change PS more than CFS There are several studies to support this theory (e.g. Renkin et al. 1966).

When the peripheral resistance was increased six-fold by a precapillary obstruction induced by intravascular injection of microspheres into a vasodilated preparation, Rippe et al. (1978) found CFC to decrease to approx. 45 %, whereas PS decreased to less than one fourth of its control value The PS decrease obtained is of the same order of magnitude as the maximal decrease seen to follow sympathetic adrenergic vasoconstriction (Renkin and Rosell 1962, Renkin 1967) However sympathetic adrenergic vasoconstriction is known to subside soon after the onset of hemorrhagic hypotension (Mellander and Lewis 1963 Lundgren et al. 1964) and leads, per se only to a smaller initial and short-lasting reduction of CFC (e.g. Cobbold et al. 1963 cf. Celander and Folkow 1953).

If the effects of hemorrhage were to be similar to those produced by microsphere injection or sympathetic vasoconstriction, one would possibly expect, in comparison with CFC, a disproportionately large or in the latter case, only an early and transient reduc tion of PS. However in the present investigation, a more moderate and sustained decrease of PS was found, the magnitude of which (50 %) is essentially the same as that reported earlier in similar studies (Appelgren 1972, PS, Baeckström et al. 1971 CFC). One possible explanation may be that there is, in acute hypovolemia, an almost total cessation of exchange processes in a certain number of capillaries, filtration and diffusion would then be more similarly reduced and both in proportion to the number of obstructed capillaries. Such a condition would mimic, at least during initial stages, an all-or-none ac tion of individual capillaries rather than a graded activity of vasomotion (cf Renkin 1969).

In the present study acute hemorrhagic hypotension was seen to lead to a decrease in PS for Cr–EDTA The first measurements during the hypotension period were carried out when at least one hour had elapsed since the onset of bleeding. Consequently the data do not permit any conclusions concerning the exchange capacity during the earlier stages of the hypotension period.

In comparison with the results obtained from a small number of experiments on regional arterial hypotension the reduction in PS of ^{51}Cr–EDTA by the indicator dilution method was less equivocal and statistically significant when first measured between one and two hours after the onset of bleeding. The magnitude of the PS reduction was the same as when measured by the local clearance method.

2. The activated state with a variable blood flow

Injury Ten experiments were carried out to study the changes of blood flow and PS for Cr–EDTA which were produced by a prolonged and widespread traumatization of the musculature of the dog's hind leg. In five experiments, the blood flow was found to increase by 100 – 168 per cent when measured within the first few minutes after the injury the flow subsequently returned to control level during the following hour In the other experiments, the blood flow decreased by 40 – 68 per cent and remained somewhat low in comparison with control. In most experiments (8/9 one excluded), PS was found to decrease This is in contrast to normal skeletal muscle where a coordinated, adaptive response to increased metabolism is seen to take place characterized by a simultaneous increase in blood flow and in capillary filtration and diffusion capacities (Lewis and Mellander 1962 Cobbold et al 1963 Kjellmer 1965 Renkin et al 1966) At metabolic vasodilatation produced by intermittent muscular contraction the increase in flow leads to an increase in PS This was observed by Renkin et al. (1966). They also found that the return of blood flow towards control level, after cessation of the muscular work, was less rapid if perfusion rate was low in relation to the metabolic load.

Data from several earlier studies dealing with trauma indicate that injury is likely to induce a local reaction which includes vasodilatation and disturbance of capillary blood flow (e.g. Landis and Pappenheimer 1963). Metabolic factors are known to influence the vasomotion of tissues and are capable to dilate the blood vessels, particularly the segments which are situated proximal to the capillaries. The metabolites thus counteract the contraction of precapillary resistance vessels and sphincters which may be produced by various mechanisms e g. sympathetic adrenergic activity If metabolic events resembling those occurring e.g. during and after muscular work would appear in connection with injury the vascular response to injury might be expected to display some similarities with the one produced by metabolic vasodilatation under more normal circumstances, such as muscular work.

In the present series of experiments it was not possibl hanges of blood flow and PS during the period when the injury was prod bility cannot be excluded that the blood flow response during this stage than what is indicated by the measurements made during the first ter the trauma. If production of vasodilating metabolites is a regular fe the period the injury is produced there are reasons to expect an increased flow at nporarily during this period.

Liu (1968) and Lewis and Lim (1970) found that the relative blood flow increase after trauma was markedly reduced or absent in animals subjected to sympathectomy. This could indicate that the response to trauma may depend on the antagonistic nature of sympathetic adrenergic nerve activity and vasoactive substances, released by injury (cf Green 1961).

In the present study carried out on a large innervated vascular bed, an increase of total blood flow was not found to be a constant response to injury. If present, it was of short duration and rarely as large as one expected from a total inhibition of vasoconstrictor nerve activity or from a moderate metabolic vasodilatation. The mean value for the increase in total blood flow was 36 per cent in the immediate post traumatic period. In similar experiments, Lewis and Lim (1970) found, at an average, an initial 50 per cent increase in total blood flow, also measured from the total venous return in the iliac vein. Sandegård (1974) found a short-lasting, immediate blood flow increase of more than 100 per cent in the external iliac artery following the same kind of injury. For technical reasons, the blood flow in the iliac vein could not be measured simultaneously in the first few minutes following injury. The comparatively small increase in blood flow may indicate that the blood flow is rapidly regulated or, to some degree, underestimated.

Studying the blood tissue exchange during metabolic vasodilatation, Renkin et al (1966) found a sustained increase of PS during vasodilatation only when the blood flow was kept high and adequate for the metabolic load. If the blood flow was kept at a lower rate, PS all the same reached a maximum after half a minute during the period of muscular contraction (90 – 120 seconds) but it immediately started to decline towards control level while resistance to flow remained markedly decreased. After prolonged stimulation at low flow, the PS response to muscular contraction could disappear entirely, while the resistance response appeared to be unaffected. The authors postulated that, at very high levels of metabolism, capillaries with very low s/q ratios may open up for an excessive flow, leading to a PS fall due to a deteriorating, i.e. a non-adaptive, distribution of capillary blood flow.

The present data reveal that PS may decrease during vasodilatation produced by injury. This finding could therefore indicate that the blood perfusion is, in the injured tissue, pathologically distributed and, possibly, inadequate in relation to the metabolic demand.

The metabolic demand of the injured muscle tissue may be considerably increased despite the variable response in blood flow and the predominant decrease in PS the changes in these three parameters do not necessarily run in parallel in a pathophysiological situation. Thus, when blood flow is adequate in relation to the metabolic load precapillary sphincters are seen to respond in an adaptive manner in order to distribute the capillary blood flow uniformly At inadequate flows, however there may develop an inability for the sphincters to distribute the blood evenly among the capillaries. This could explain a rise in PS being smaller or more short-lasting than expected at a certain degree of vasodilatation, even if the sphincters initially may seem to be more sensitive to metabolite action than precapillary resistance vessels (cf Mellander and Lewis 1963).

The decrease in PS should also be considered in relation to rheological factors. Following the kind of standardized injury which has been presently employed, local accumulation of erythrocytes eventually appears intravascularly and may be able to impair the capillary blood flow after approximately one hour at least at the post-capillary level (Knisely et al 1947 Bigelow et al. 1949 Gelin 1956).

Other kinds of corpuscular obstruction (platelet aggregates, leucocytes) may possibly appear much earlier The ability of the leucocyte to move along a capillary seems to be much reduced in hemorrhagic shock and may be directly related to the level of prevailing perfusion pressure (Bagge et al. 1979) It is not, as yet, known whether a similar capillary obstruction may occur in the capillaries of a musculature which has been subjected to severe contusion. The decrease in PS which is seen to occur early after injury cannot therefore at present, be accounted for by any known capillary obstruction by blood corpuscles, although such a mechanism deserves further investigation.

3 The activated state with an increased blood flow

Acute normovolemic hemodilution. The rapid single-injection multiple-indicator method was employed in fifteen experiments. In six experiments a local clearance method was simultaneously used to study the transport of the same water-soluble indicator (Cr–EDTA) in the opposite direction across the capillary wall

An increasing amount of whole blood was replaced by dextran 70. The change in plasma flow at two levels of hemodilution (hematocrit 25 – 30 and 10 – 12 %) was estimated by measurements of the total venous return and, for the local clearance method, by measurements of the Xenon clearance Similar flow data were obtained from both methods, indicating an increase in plasma flow for each step of progressive hemodilution (55 and 107 %) The increase of total blood flow which is seen to follow hemodilution is expected as a consequence of the decrease in whole blood viscosity In a previous study total blood flow was found to be twice as large after extreme normovolemic hemodilution (Hct 10 %) as in the control state whole blood viscosity and total peripheral resistance were at the same time reduced to half their control values (Messmer et al. 1972). The

capillary blood flow estimated by local ^{133}Xenon cle[illegible] [illegible]und to increase three-fold compared to control However it appeared as i[illegible] [illegible]fusion capacity remained unchanged in hemodilution the increment [illegible] [illegible]vas not accompanied by any change in PS for ^{51}Cr–EDTA, which was sin[illegible] [illegible]asured by the local clearance method.

In the present study the total flow of plasma incre[illegible] [illegible]d two fold after hemodilution to a hematocrit of 10 – 12 % indicating a smaller [illegible]hange in total blood flow than was observed in the study previously mentioned. Some of the difference may depend on the blood flow response to the surgical preparation which was more extensive in the present study Control flow and PS were both lower in the present investigation.

PS for Cr–EDTA, when estimated by the indicator dilution method, increased for each reduction of the hematocrit level the values (based on plasma flow rates) were 65 and 122 % When the change in PS for Cr–EDTA was estimated by the local clearance method, much smaller changes were registered At the first level of hemodilution there was an increase, at the second level a decrease in PS (13 and 12 %) both values, however were statistically insignificant.

It is interesting to note that the indicator dilution method and the local clearance method appear to be unanimous when recording the changes in blood flow and in PS during hemorrhagic shock whereas diverging results emerge for PS during hemodilution The control states do not reveal any dissimilarities in these two series of experiments (cf. Tables IV and VII and Figs. 10 and 15 mean hematocrit = 45). The two groups of data obtained by the local clearance method during hemorrhagic hypotension (decreased Q decreased PS) and normovolemic hemodilution (increased Q unchanged PS) are both in concord with previous findings (Appelgren 1972 Messmer et al. 1972) comparable data are not known to exist for the indicator dilution method.

The response in PS measured by the indicator dilution method appears to be uniform. The control measurements indicate in comparison, low levels of plasma flow within a flow range from which the changes in flow per se are expected to be highly influential on PS (e.g. Appelgren 1972b, Rippe et al 1978)

The response in PS, measured by the local clearance method, may be explained by the fact that control measurements of plasma flow extend into the flow range where changes in flow elicit comparatively moderate changes in PS For each measurement an intramuscular injection of isotopes with a separate location was made which may also contribute in obscuring the results by increasing the spread of values in the control state This reasoning is supported by the wider confidence limit obtained for the change in plasma flow by the local clearance method (hemodilution, level 1 101 – 239 % = 138 % level 2 126 – 311 % = 185 %) in comparison with the corresponding confidence interval for the change in plasma flow obtained by measurement of venous return (level 1 1[illegible]7 – 184 % = 57 % level 2 167 – 276 % = 109 %). Cf Figs. 14 and 15

r

4 Increased venous pressure

Capillary filtration capacity (CFC) and the capillary permeability surface area product (PS) are commonly found to be reduced when the transmural pressure is elevated (Mellander et al 1964 Johnson 1964). This reaction is generally accepted to be due to a myogenic response to elevated smooth muscle tension with a closure of precapillary sphincters. A similar "teleologic" mechanism, by reduction of CFC has been suggested to explain the protection against oedema formation in the lower limb in man in the prone position (Mellander et al. 1964).

The blood-to-tissue transport at increased venous pressure has recently been studied by Åberg (1973) who found the extraction ratio raffinose/sucrose to approach unity in experiments on the cat hind leg. Åberg explained this by an acceleration of transport caused by convection during the filtration process

However Fleming and Diana (1976 1977) reported only a minimal effect of venous pressure elevation on blood-to-tissue transport of small molecules. They used a single-injection multiple-indicator dilution and isogravimetric technique in dog hindlimbs vasodilated with papaverine

In the present study extraction of ^{51}Cr–EDTA, did not appear to be affected by elevation of venous pressure more than expected from a small flow reduction at constant PS This was the case whether venous pressure was temporarily elevated or continuously elevated for several hours. The findings are in keeping with the hypothesis that net transcapillary fluid movement does not to any considerable degree affect the transport of small molecules through the capillary from *blood to tissue* A reduction of the capillary bed (S-reduction) at increased venous pressure – such as described by the authors mentioned above – but compensated for by a facilitation of blood to tissue diffusion at net filtration of fluid cannot be excluded with the present experimental design. However the present results are in agreement with the observations by Fleming and Diana (1976 1977).

In contrast, Lundgren and Mellander (1967) reported an increased transport *from tissue to blood* during net transcapillary fluid movement regardless its direction. In this state concentration gradients in the tissue may be of greater importance than in transport from blood to-tissue (Crone and Garlick 1970 Dahlberg and Lewis 1976). Such concentration gradients may be more or less dispersed by an increased paracapillary flow

The extractions of the lipid-soluble ^{125}I-antipyrine seemed to be maximal under control flow conditions and were not affected by short or prolonged increase of venous pressure

SUMMARY AND CONCLUSIONS

The blood tissue exchange of hydrophilic small solutes (Cr–EDTA, Iodid soluble substance (Iodoantipyrine) has been studied in the skeletal muscle

The single-injection multiple-indicator method and to some extent the method were employed

The vascular bed in the skeletal muscle was not initially controlled by any vasodilating procedure A great deal of the differences that were demonstrated between the capillary beds of individual preparations may be due to this fact which also constitutes a limitation with regard to the interpretation of the results.

The main parameters were blood flow Q and (for lipid-insoluble indicators) extraction of indicators, E, and capillary diffusion capacity PS. The interrelationships of these parameters in the control state and their response to changes induced by regional arterial hypotension, hemorrhagic hypotension injury normovolemic hemodilution and graded obstruction of the iliac vein were studied. The evaluation of the results was greatly facilitated by a computerized statistical analysis of the entire group of control data and of the data obtained in each series of experiments.

The extraction of I-antipyrine, approx. 0.80 was independent of changes in the blood flow rate and in any of the activated states.

At decreased blood flow PS for Cr–EDTA decreased. The extent to which rheological factors, such as capillary obstruction by corpuscular blood elements, may have contributed to cause this decrease, could not be evaluated.

After a period of one to two hours of hemorrhagic hypotension at an arterial blood pressure of approximately 50 mm Hg, a 50 % reduction of PS for Cr–EDTA was estimated. The decrease in PS was the same and statistically significant, for both the indicator dilution method and the local clearance method. The results are in accordance with previous studies on capillary blood flow following acute hemorrhage

As a result of injury PS for Cr–EDTA usually decreased markedly and tended to rise during the following hour The change in PS measured by the indicator dilution method, seemed to be independent of the response in blood flow and was present from the very beginning of the observation period even in those animals which initially displayed a considerable increase in blood flow The results indicate that the local distribution of capillary blood flow may severely and rapidly deteriorate when skeletal muscle is injured by powerful contusion and that the local blood flow in the anaesthetized dog, may decrease or increase as a result of such an injury

A successive increase in plasma flow was seen to characterize acute normovolemic hemodilution. Portions of the blood volume were replaced, step by step by equal amounts of dextran 70 a colloid plasma substitute The increase in plasma flow exceeded 50 % at a hematocrit level of 25 – 30 % and at a level of 10 – 12 % the increase from control was

twice as large The values for the changes in flow were nearly identical for the indicator dilution method (total venous return) and the local clearance method (Xenon clearance). Data obtained by the former technique indicated an increase in PS for Cr–EDTA. In contrast no increase was registered when PS was measured by the local clearance method, used in six of the fourteen experiments. The reason for this discrepancy is not clear but may be sought for in the larger spread of values for changes in flow as registered by the tissue clearance method

At graded compression of the iliac vein the venous return was partially diverted into collateral veins. The increase in venous pressure did not seem to affect PS for Cr–EDTA.

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Spleen

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SUBJECT INDEX

COMMUNICATIONS

1

Kiil, Fredrik. (Institute for Experimental Medical Research, University of Oslo, Norway) CARDIAC PERFORMANCE

A main goal of our studies on both conscious and atropinized anaesthetized open-chest dogs has been to apply to the intact heart concepts derived from studies on isolated papillary muscles. An ultrasonic technique for measurement of cardiac diameters has been extensively used by many investigators; during ejection the diameter of the ventricular cavity is reduced whereas the myocardial walls thicken. This technique can therefore not be used for estimations of fibre shortening. T obtain more direct estimate of variations of mean changes in myocardial fibre length, ultrasonic elements were sewn into the anterior wall of the left ventricle centimetre apart and the distance between them, the myocardial chord length (MCL) continuously recorded. When the maximal MCL coincide with start of ejection and minimal MCL with completion of ejection the recordings are representative for the whole myocardium; high correlation was obtained between stroke volume and the difference between end-diastolic and end-systolic chord estimated from MCL recordings. Such recordings have led to the following conclusions:

1. A rise in afterload (induced by proximal aortic constriction) reduces myocardial contraction so that end-systolic MCL increases, but is without effect on end-diastolic MCL. Afterload may also be increased by ventricular expansion at constant systolic left ventricular pressure. The increase in end-systolic MCL caused by increased afterload is large at low inotropy and may not be significant at high inotropy There is no sign of "homeometric autoregulation"
2. A rise in preload results in increased end-diastolic MCL and may reflect increased circulating blood volume during elevation of systemic blood pressure rise in preload is consequence of redistribution of blood volume towards the heart. Because of increased end-diastolic volume and almost unchanged end-systolic volume rise in systemic blood pressure may at high and constant inotropy increase stroke volume.
3. Variations in heart rate between 40-80 beats/min have no effect on end-diastolic MCL and stroke volume. A rise in heart rate above 80 beats/min reduces end-diastolic MCL because of curtailed filling, but exerts little effect on end-systolic MCL. A rise in blood volume increases stroke volume more at low than at high heart rate. When control end-diastolic volume has been restored by raising heart rate control end-systolic volume is also restored. A rise in heart rate exerts therefore no inotropic effect; variations in heart rate and blood volume affects stroke volume only by altering preload.
4. A rise in inotropy reduces end-systolic MCL but has no effect on end-diastolic MCL if heart rate is kept constant. The reduction in end-systolic MCL and increase in stroke volume induced by raising inotropy is equal at any heart rate.

Thus rise in inotropy reduces end-systolic volume and is without effect on end-diastolic volume. Conversely; rise in preload induced by increasing circulating blood volume or reducing heart rate towards 60 beats/min, increases end-diastolic volume but exerts little effect on end-systolic volume. Therefore, inotropy and chronotropy are independent variables in the regulation of stroke volume.

2

Rowell, L.B. (Departments of Physiology and Biophysics and Medicine University f Washington School Medicine Seattl USA) REFLEX CONTROL OF THE CIRCULATION DURING EXERCISE

Human circulatory responses to exercise are now well documented. But the stimuli the effect (a) the [illegible] matching heart ate (HR) and cardiac output (Q̇) to V̇O₂ (b) the rise in arterial blood pressure (BP) and (c) vasoconstriction in non-working regions are still unknown. The following have been postulated to provide the exercise timulus (1) humoral agents released from working muscle (2) arterial baroreceptors (3) arterial chemoreceptor ( ) central venous chemoreceptors (5) irradiation of nerve impulses from the motor centers (central command) (6) muscle mechanoreceptors and (7) muscle chemoreceptors. With (1) ( ) above presumably ruled out, recent attention has focused on (5) (7). Central command (5) probably p ays minor role. Studies employing differential nerve blockade and nerve recordings (in cats and dogs) support the muscle mechanoreceptors (6) play no role ince 1886 when Zuntz and Geppert first postulated their role investigators have tested the dea that muscl chemoreceptors (7) could provide the "exercise stimulus" by sensing certain chemical changes within the muscle The early demonstrations by Alam Smith and Asmussen Nielsen that pressor response accompanies contraction of ischemic muscle form the keystone of this thinking The pressor response associated with isometric exercise may be related phenomenon In man and other species interruption of sensory nerves rom contracting muscles wil abolish this response which is cats) appears to be carried by the fibers

Recent f orts to character ze further the ischemic muscle pressor response (IMPR) [illegible] ted by 10-30 sec periods f leg occlusion during exercise with occlusion continued for min after exercise showed the following during min of post exercise occlusion (a) IMPR is graded in direct proportion to the degree of muscle ischemia and also (b) is direct proportion to the ischemic muscle mass ( ) IMPR usually results from higher than normal Q̇ during occlusion (d) IMPR is accompanied by marked vasodilation (baroreflex) he in cases where Q̇ and HR are reduced IMPR is maintained by vasoconstriction (e) $\dot{V}_E$ and end-tidal CO_2 are unaf ected by post exercise muscle ischemia (f) IMPR is increased by maneuvers that raise baroreflex sensitivity (reduced thoracic blood volume) and reduced by maneuvers that lower sensitivity (increased thoraci blood volume) (g) dif ferential blockade of sensory nerves (peridural anesthesia) abolishes IMPR but does not block normal cardiovascular respiratory responses to exercise without occlusion

These above results support that (1) the baroreflex helps to set the levated level of BP during IMPR. (2) There is no drive from muscl chemoreceptors to respiratory centers (3) Normal exercise responses may not require functioning -fibers or alternativel (4) large (unblocked) nerve fibers may also conduct muscle chemoreflexes or (5) muscl chemoreflexes may only be acti vated when muscle perfusion falls below some critical level and then they somehow raise the baroreflex oper ating point

3

Omtvyit K,M[1] Haug E[2] Iversen, J -G[1] Sand O[1], Halaas E, Halaas O, Lystad, E and Abraham, A[1] (Institute of Physiology Hormone and Isotope Laboratory Aker Hospital[1] Institute of Medical Biochemistry Veterinary College of Norway Oslo Norsk Hydro Institute for Cancer Research, Oslo 3 Norway) REGULATION OF PROLACTIN (PRL) SECRETION AND SYNTHESIS.

PRL is polypeptide hormone which is synthesized and secreted by anterior pituitary cell T study the function of lactotroph cells we have used lonal strain f rat pituitary tumour cell which spontaneously secretes PRL in vitro. These cells respond to the hypothalamic releasing hormone TRH and to 17β-oestradiol Both hormones stimulate PRL production but they exert their effect through different molecular mechanisms TRH induces half maximal stimulation of secretion and synthesis t 0 3 nM and 3 0 nM respectively The f llowing parameters have been measured and are believed t be of importance for initiating the biological ffect 1 ³H-TRH binds rapidly to the cells at 37°C and 2/3 of maximal binding occurs within 30 sec The binding of ³H-TRH is specific saturabl and reversibl Maximal binding equals 30-400 f-mole ³H-TRH/mg cell prot in. Two different high affinity binding ites with apparent Kd, of 0 3 nM and Kd, of 25 nM are present 2 Electrophysiological studies show that hese cells exhibit spontaneous action potentials whose duration and frequency are increased after addition of TRH 3 Addition of TRH t monolayer cultures causes transient increases (2 5 fold) in cellular cycli GMP and in cyclic AMP (2-3 f ld) The concentrations of TRH that are needed for half maximal stimulation of cGMP and cAMP levations are about 3 M and about 70 nM, respectively Phosphod esterase inhibitors (theophylline methylxanthine) stimulat secretion as well as synthesi of PRL and increase the concentrat on f lic nuc eotides 5 Dibutyryl cycli AMP and tyryl cyclic GMP stimulate the sy thesis of PRL but only dibutyryl cyclic AMP is able to enhance PRL secretion 6 Cells cultured in calcium free medium show reduced spontaneous secretion of PRL and TRH is unable to stimulat hormone secretion When cyclic nucleotides are measured after TRH treatment in the absence of calcium, cAMP elevations are still observed but no increment in cGMP formation occurs 7 After preloading of the cells with ⁴⁵Ca TRH induces an increased efflux preceeding PRL secretion. 8 The cell contain two different cAMP phosphodiesterase activities with Km of 0 71±0 05 μM and of 20 2 8 μM respectively Treatment of the cells with TRH increases V max of both enzyme activities with time-course similar to that of cAMP levation. 9 In cell homogenat cAMP dependent and independent protein kinase activities are demonstrated. The cAMP dependent protein kinase is half maximally stimulated by TRH at about 25 nM. 10 The binding of ³H-17β-oestradi l to monolayer cultures of 4°C is rapid (half maximal at 2 hr) saturable specific and reversible ³H-17β-oestradiol radioactivity accumulates in cytoplasma at °C but is translocated int the nucleus when the temperature is increased to 37°C The cytoplasmic radioactivity is found to be associated with macromolecules, sedimenting in sucrose density gradients as 8S substances and with nuclear 5 S macromolecules 11 The combined treatment f TRH and 17β-oestradiol shows additive ffects on PRL synthesis 12 TRH pretreatment reduces the number of binding sites for ³H-TRH as well as th TRH-induced levation of cAMP while 17β-oestradiol pretreatment doubles the number of available ³H-TRH binding sites 13. The mode of action of TRH and 17β-oestradiol on PRL synthesis is studied further in cell free wheat germ translation system. Possibl changes in PRL specific mRNA activity associated with stimulated hormone synthesis are examined and compared to the importance of lterations in cellular receptor ites for PRL induction after treatment with TRH and β-oestradiol

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Meyerson, B.J (Departme t f Medi l Pharmacology U ive ity f Uppsala Swede) NEUROPEPTIDES AND BEHAVIOR

Neuropeptide have dual rol in l tion to beh i proce s ( ) as hypothalami hormone link between environme t l imuli and the sec tion f t oid hormone that con rol behavi and (b) pos ibl di ec ff on euronal mechanisms impli ted i behavi

The p en ynopsi i mai ly conce ned with the di ec ffects f neu opep ide i dif f t behav o processes Th ilabl i f mation on ne ropeptide and behavi be di vided i three tego i

1 The pecifi ffec on spont neou beha io i beh i bei g normal cons itue t f the imal epe ire uch as feeding so- i l i t ior xual behavi

2 Th mod l t ry i flue ce f drug-induced beha io p tt rn The pep de i self l ck ffects but f litat inhibi the pons f psycho ropi ge

3 The ff ct on l rned re ponse Mos di have been ied ou on so ll d ne g tively condi ioned beh i The animal l rn o void neg ve ff by pr di g t cert i mul Ne opep id have bee f nd t i flue diff p of thi p oce

Cholecy toki i ha been own dep food i t k mpl t ll imul t d k ng d LHRH eportedly f il ce lenesis f copulat ry beh i a fer l Neuropeptide whi h mod l d g ad cel beha i rs i TRF M.F I wh ch f il tal espon es t DOPA T i now te re docume tation howi g th pept de ch opre i MSH/ACTH f The i is i thine ff t l nei re po y wh ch uggest that neuropeptides might influence beha i by acting on processes which re more common i teg l part f the machinery producing behavi Certai gents f peptide tructure migh t rn out to have selective action on ment processes rel ted to vigilance and at ten on Behavi i depende t on contact between th individual and pecific environme t l items The ability to dire t mental activity must be very esse tial pre equi ite for the furthe proce ing of ce tal behavi Learning nd memory ctiviti an lso be the possibl foci for the ctio f uch peptide Thi i f course f obvious ignifi nce Our behavior i particul ituation i the i t grated function f innate nd acquired mecha isms Any difficulty to detect recogni to consolidat retai and trieve i f rmation should be very import t f ou beh i

The ffect f β-endorphi on xplo ry and socio- xual beh i patt rns will be desc i bed nd di cussed with reg d to wh t i id above

I pit of the modern very sensitive chemi l y the ignifi nce f neuropeptide for mental ctivi y must lso be based on r levant function l me sur Incr ed i igh i t th physi logi l be ring f ne opeptide require improved behavi l t chnique with l rly defi ed behavi l items be me ed (f f nce ad f the di cu ion ee Meyerson, B J Hypothalami Hormone d Beh io Medi l Bi logy 1979 i p)

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Bergström, R.M. (Institut f Physi logy University f Hel inki Finland) INFORMATION PROCESSING IN NEURAL SYSTEMS

The abstract sta i tical inf rmation theory takes no count for the cons rvation f energy and matter in he communication ystem This might be the use f its l ok f uccess in xplaining neurophysi logical processes, pe cially higher orde information processing An alternative i the physical information theory (Brilloui , L Science and inf rmation theory New York, Academi Pre 1963) which connects information flow with flow f entropy and distribution f energy and matt In neural systems proce sing f information i proces ing f di ributions f ignals and thei material ubstrat the ynapse

Whereas the coding mechanisms in ingle neurons are rathe well known there are great difficul ies concerning comple networks This i onsidered as being due t lack f knowl dge f the wiring geometry f mi rocircuits (Edelman, G M and V B Mountcastle The mindful brai Cambridge MIT Pre 1978) Since however thi geometry i not stable, magnitude re needed f description f ariation f sy napse due plastici y inhibition et , together with variation f ignal activity (code parameters being numbe f units (M) numbe f hei ff rent ynapses (N) amount f ignals ( ) and their orde ( )) Attempts for pecification f uch magnitudes have been made (Scott A C Neurophysics John Wil y Sons In 1977) Thes magnitudes may be f the main f rm Q NV defini g the ffect of neurons with N sy napses and verage pike frequency I I 1 possibl t define concept ch as neural mi rost (f lements) and macrostate (f averages) with corresponding levels f inf rmati processing (Prigogine I Physics Today 1972 25 23) As an xampl an integration f micros 0 over M neurons define macrosta f which the ntropy distribution ( ) and hence the information pacity pos sesses maximum t medium l vel f verage pike frequency

There also exists the pos ibility of defining the neural macrosta as ubjective psychological ta (measurable only with psychological methods) nd the neural micro at as an obj ctive physiological tate (measurabl only with physiological methods) (Bergröm R.M In Ad ances in Psychobiology (Eds G Newton and A H Riesen) John Wiley 1972) As predicted by thi definition the calcul tion f average of lementary neural vents should reveal the same mathematical information functions the direct mea urement f he corresponding psychologi al vents As an xample of his imila paraboli dependence (inverted U-shaped functions) f inf rmation processing apaci y on the level f xcit tion can be *obt ined both by calculation from the physio*logical (micros ta) paramet rs f the neural ystem (Bergström R M and Nevanlinna O In- t rn J Neurosci 1972 4 171) and by direct psychological (ma rost t) measurement (Hebb D O The rganization f behavio Wiley New York 1949)

The definition f highe rde conscious information processing as macrostat processing possesse no difficulties from thermodynami point f view as regards the int raction between physiologi al nd psychological vents Nor i the lution f he mind-brain problem inherent in this view loaded with difficulties contained i the mat riali i phenomenologi parallelli ti and indentisti solutions

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Bechtereva N.P. (Department f Human Neurophysiology, In titute for Experimental Medicin Acad.Med.Sci.USSR, Leningrad USSR) NEUROPHYSIOLOGY OF MENTALITY

When studying the multiunit activity of neuronal populations of the human brain (in the course of diagno is and therapy) with the aid of original, adapted and standard mathematical technical approache the areas were revealed in which perception and reproduction of words were correl ted with local spatial temporal rearrangement f the multiunit activity - the patt rns The semantic commonness f words is characterized by presence of common lement in their patt rns the group equence of discharges with fixed int rvals The specific character of the patterns and of some f their lements suggested the standard *computer search techniqu* using the formers as standards Accumulation of individual banks of the patterns-standards for different words made it possibl to study the neurophysiol gical dynamics devel ping during the long-t rm memory activation in the course of generalisation mental conclusion, decision making The local patterns in multiunit activity are virtually different in diff rent links of th system fo maintenance f mental activity which indicat the spread of the neurophysiol gical organization of mentality and unequal contribution of diff rent brain structure in thes phenomena. Local patterns f the ame word in different cerebral areas and patterns f words belonging to the same emantic fi ld in the same are possess also certain common lement which seem t b europhysiological expression for system-forming facto

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Gust f son, J A (Depa tments f Chemi try and Medi l N t ition K rolinska Institute Swede)
HYPOTHALAMO-PITUITARY REGULATION OF LIVER META BOLISM

Sex diff renc exi t i hepati drug and te- roid metabolism in the rat nd pos ibly i a numbe of othe species including man Sex diff ences in drug and st roid metabolism a not seen before 30 days f age but the ignal fo the diff ences i give soon afte birth Thi ignal i thought t be t tost rone t les t parti lly acting vi conversion to estrog n on n impri ting centr i the b i

The pres ion f the imp i ting i medi ted via the pituitary gland which only in the f mal t secret fact (t rmed femi i ing fact) that maint ins the femal type metabo- li pattern The ffect f femi i ing facto i i pa t on hepati cytochrome P-450 Sex t roid in the adult pe iod ffect hepati te roid and drug metabolism by n effect on femi ni ing f ctor secr tion The nat re f thi hypophyseal factor i yet unknown but it doe not appear t be one of the known pituitary hormone

The impri ting ce tr ha been shown to re side in the hypothalamus Frontal d ff enta tion t the trochi smati and suprachi smati level alt i compl t femi i tion of hepatic t roid metabolism i mal ts Such an ffect i lso seen when l ion invol ing mai ly the ant ior pe i ent icula hypothalami and the supr hi smati nucleus re per- formed i mal t No ffect re seen in an logou l sions i femal t i ny f the es tudied

The finding sugg t that egion incl ding the t ior hypothalami periventricula the suprachi smati nucleus and adjacent are is involved in the control of hepati t roid metabolism It i post lated that the neu- ronal cell bodie that produce a factor with an inhibitory ffect on the ect tion f femini ing factor have their origins in thi rea of the hypothalamus or alternatively, may send axons through this re to the basal hypothala- mus and thus directly o indirectly influence the ante io pit itary gland

With liver membrane from femal rats, 6 8% of totally added ^{125}I hPrl i specifically bound whereas in mal live memb ne fractions the specific Prl binding i very low o absent Ante- ior hypothalamic deaff rentation at the retro- chi smati level in mal r ts increase the hepa tic hPrl receptor conce tr tion to the femal level 3 4 day following the ope tion A tran section rostr l to the suprachi smatic nucleus ha no ffect on the concentr tion of hPrl re- ceptor i male animal These lts demon- tr te that the numbe f hP l ecept rs i regu- l ted by the hypothalamo-pit it ry system The receptor inducing pituitary facto might be re- l ted t the femini ing factor

Schou, J (Dep tment f Pharmac logy Un ve ity f Copenhagen 2 J liane M i 'ej DK 2100 Copenhagen O) MECHANISMS OF AD- VERSE DRUG R ACTIONS

Adve se drug ction cove all ction whi h un nted i re pect ve f thei ch ct They may i he be well known toxi re ction un xpected and ly occuri g action i hout pe if dose dependency

As ugge t d by Mitchell t l they an be di id d i 1 Exagge ed unwant d drug ction 2 T i d ug ct on cell cel drug d ced neopl i d drug ll gy d f lly one 3 D ug ction f unknown logy

Spe i l focu ill be on t l on h p be wee phy ico hemi l ch ct i i and th occu ence f d ff ct

High lipid l b l y (HL.) re ry f drug eff ert re t CNS but HLS l ffi i y ll he l g d ...ubl comp tment the body d low ren l l ce due t ub l b r on con equence No FLS ance ave gh ffi ity t h live d y boli ed ith or bl r Th d f met bolit ard p oblem f f r t ll ill be di cu ed

E pe ially long t m ct a- geni y mu gen ity d p oduct on i ity h ve bee gi en m once du g he l y I ce l ice ll be given t l th l l f f y be t i i bef i ct on f ew d g b d upon h cu he hod f f diet ve icologi l i g on

E...al J R chell W
en P ... J ll and
Gill tt Toxi Drug R action in Concept i Biochemi l Pharmacology Eds Gill tt & Mitchell Sp inge Ve lag Be li 1975

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Ursin, H. (Institute f Psychology Universi ty of Bergen Norway) STRESS DEFENCE AND COPING IN MAN AND ANIMALS

Stress is an unfortunate term used partly for stimuli partly for response This response i identical to activation Sustained activation i known to produce gastric ulcerations myocardial infarctions and hypertension in several species Sustained activation occurs in threatening and dangerous ituations which the indi idual is unabl to control It also happens in difficult ituations with conflicting cues Conversely activation is reduced when the individual is mastering the task and learns that he i mastering This proces is called coping In humans perceptual defence also dampens activation but this dampening may interf re with mastering the task the individual may not realize the dangers involved

Multivari te analyses have identified three specific endocrine activation systems They relate differentially to psychological traits Cortisol esponders have high defense mechanisms and poor perf rmance in dangerous tasks The d fence mechanisms may be the reason for the poor performance but these mechanisms defend ag inst realizing the poor performance and may therefore help the indi idual against psychosomati disease Catecholamine responder have high reactivity and good performance Thi reactivity may be handicap when the task is unsolvabl This may explain the postulated relationships between this personality type and cardiovascular disease Testosterone responders may have particular difficulties

These factors do not represent types in the lassical sense The factors occur in all subjects but to varying extent Further work with this model requires analyses of multipl hormone systems with multivariate techniques

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Pa sonen, P K (Department of Pharmacology University of Hel inki Finland) TAURINE IN PLATELETS AND HEART TISSUE

Tau in $(NH_2CH_2CH_2SO_3)$ (T) i an amino sul phonic acid pr sen in ll animal species In animal high amounts f T (2 40 mol/g) re found in the brain tin heart plat lets and ilk Apart from its l in bil salt f rmation no physiologi l function i known

T It has some f th characte i tic f inhibi ry neurotransmitte it inhibits ex perimental seizures and ay have an iepilepti action I he has t T prevent rrhythmias induced by adrenaline cardia glycosid and i h lps he guine pig heart to bea whil it i pe f sed with Ca^{++} free medium All the ffects may be due to T produced memb ane tabili ing ffect by the regulation f cell membrane permeabili y to ion (see S F Oj Prog P r col 1977 I 1; A. Barbeau R. uxtabl (eds) ri and rcl p d Raven New York 1978)

W have used pl lets rodel for amin rgi neurons fo many ye rs (P K Paasonen F t pr P ar 1972 338) and lso f und h they cti ly accumul t T ((L Ah ee Arc J Phar 1 1974 41 245) Like in nervous i sue this uptake proces f l l ts i tereo pecifi and e quir rosi i ly charged p imary amino group and ion zed sulphoni acid group Mos f the pl l T we found in the supernatan f ction fte high speed entrifug tio I the h T repres abou 50 % f the free amino acid pool There i experimental evidence both f be synthesi nd uptake of T by the hear Th heart T level f anir l ar no ffected unde ang f st condi ion However the concentr tion f T is le vated twofold in the fected left ventricles f patients dying f congestive heart f ilure Similar change have been observed in dog and with ardiac hypertrophy These changes may be due to an incr sed T influx induced by β adrenergi stimulation (R. Huxtabl & J Chubb Sci ce 1977 198 09)

W have shown that in the spontaneously hypertensive (SH) t the content f T in cre ses both in the hypertrophied l ft ven tri l and pl t l ts I addition the plate l ts f hyperten ive SH ts accumulate in ((2 to 3 times mor f H T than the plat l ts f the control ts (M K Paasonen f I in Pl Le p 411 G. d Gaetano S Garattini (ed) Raven New Y k 1978) In the SH t the C^{++} concentration f the l f ventricle was lmost 50 % lower than tha f the control t It i po ibl that in this hypertrophied tissue the increase f T compensat the Ca^{++} decrease When the blood pressur incr se i we induced by subtotal nephrectomy and NaCl solution sol drinking fl id ther we neither an incre se in the T content f the pl el t nor in th ir uptak f H-T ((Pl te l ts we lso obtained from congest ive heart f ilure and hyperten i patients Th were no lear differences in the amount f endoge nous T bet f (ir the platel ts f both groups Accumul ed l H T than the pl te let f control

Ou recen sults ith pontaneously hyper ten ive indi the blood pr sure in crease preced the plat let-T change The i howeve no proof tha the T changes in platel ts and heart and the t ar causally rel ted in

Sigrid Jusélius Foundation)

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B strup, C, nd Nielsen M (Rese rch Labo t ies A/S F rosan 5 Sydmarken DK 2860 Soeborg and Psychopharmacologi l Research Labo atori s Sct Hans Hospital DK-4000 Roskild Denmark) *BENZODIAZEPINE RECEPTORS AND THEIR POSSIBLE ENDOGENOUS LIGANDS*

High affi ity binding f tritium labelled morphine and morphine lik drug to r t brai membranes was a deci ive advance in the cha cterisation of opiate receptor and th discovery of enkephalins and endorphins

It appe ed ecently that anothe important group f psychoactive drugs the be zodiazepine bind with high ffinity to ingl cl s f turabl ite on b in membrane of ll highe vertebr te including man

The e is good co l tion between the pharma cological ff ct f diff rent benzodiazepine and thei ffi ity f 3H-di zepam and 3H fl nitrazepam binding ite indicating tha the binding it i the physi logic l rel ant receptor upon which benzodi epine ct

Selective neuronal l ioning expe iments with the neurotoxine k i ic acid s well as investig tions f neurologi l mouse mutant indicate that brain ben odia epine receptors loc ted on neuronal pl sma memb ne Ben dia epine recept r not prese t to any m surabl xtent on gli l cell or i pe i phe l ti sues

The highe t concent tion f ben odi epine recept i huma b i i found in cerebr l cortical regions intermedi t levels ar found i midbr i and some limbi tructures whil whit matte are xhibit low level

Comprehensive lectrophysi logical evidence hows that benzodiazepine enhance GABA-ergi inhibition Behavioural and biochemical evidence mostly but not uniformly agree with this contention Benzodiazepine eceptors may be involved i these interactions ince GABA incr ase *in vitro* the ffinity of benzodi epine eceptor f 3H-di zepam and 3H flunitra epam. Several GABA-analogue including the t eolective enantiomers (+) and ( ) 5-methyl muscimol mimi GABA) and their enhancing ffect is compl t ly and electively abolished by the GABA tagonist bicuculline

The presence of benzodia epine receptors uggest that ther may be an endogenous ligand for these receptor Hypoxanthine nd inosine have been isol ted from brain and exhibit some ffinity for 3H-diazepam binding ite (K_i ~ 1000 µM) Some high molecul weight proteins (Mw 70 000 and 15 000) lso inhibit 3H-diazepam binding The most promi ing endogenous li gang however ha been isol ted from human urine and is a very potent yet unidentified inhibitor of benzodiazepine eceptors (K_i ~ 0 015 µM) Scatchard analy is of this competitive inhibitor (lled γ_1) indicat s that it diff rentiates between subpopul tions of benzodi zepine receptors

I conclusion it ppears that the may be more than one l ss f br in benzodia epine receptors At lea t one las f receptors are functionally coupled to GABA eceptors In addition an endogenous ligand(s) f r benzodi epine receptors probably exi ts

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Ljungdahl, B (Department f Physi logy and Medi l Bio-p (ve (y f Upps l Swed) RENAL HOMEOSTATIC RESPONSES

The kidney ac as he mai xcr ory rgan in he maintenance f and lectrolyt balanc Many y ems or the hi homeost i The mechanisms involved he ion homeos sys ems eal p rtly explained and he quanti ative impor ance f he ys ems ha b f he have ig ed

F ey or f he gulating sy em an have an al location an ex re l ere whe he ignal f om he eceptor dire ly or eve ouga ent f he ex l eurons y em rv en he kidney by erents nerve I i d y em group impos ibl point ecp er enal ff ct ll themselves l imulus abov d ac p lem of our p sent knowledge will be given and he ems d below

RECEPTORS AND SYSTEMS		EFFECT
Extra-renal receptors	...	ang II-p ed ff nerve ac i i
	l ...	... pred ct ... or c ...
	...	l ...
	ll (ry); ...)	
	... tal volume	ADH p od ac i ... r nerve
	...	
Extra-renal ...	... (and ...) CNS	... sympathe nerve ...
	Ang II ... rept (... renal	...

mats.

RECEPTORS AND SYSTEMS		EFFECT
Ext arenal eceptor	Ang II-recep ors i CNS	Activation f Na-recept rs?
	Autor gula ion f enal ...	Autoregul glomerul blood flow and filt tion
	Glomerul r-tubu-l b lanc	Influenc on tubul reabsorption
Int arenal endogenous sy ems	Tubulo-glomeru-l feed ba k	Regul glomerul fil ra ion at
	Hydros i and onc i p e-sure	I fluenc on glomerul f l tion and tubular bsorp ion
	Pros aglandi sys ems	Produc pros glandi

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Aukland, K (Institute of Physiology Univer i ty f Bergen Norway) LOCAL FACTORS IN THE REGULATION OF INTERSTITIAL FLUID VOLUME

As long as the intracellular volume emains constant the total extracellular volume (ECV) eflects the balance between intake and renal excretion f salt and water However the par tition of ECV between pl ma and inter titium is governed by net capillary filtration and lymph flow allowing changes in local inter titial fluid volume (IFV) in the face of unchanged total ECV Examples are orthostati edema and acute lung edema

In the case f increased microvascular pressure g in pas ive depending limb the ise in capillary pressur (P) and net filtra tion pressure ($\Delta P = P_c - P_i - \pi_p + \pi_i$ where P_i is interstitial fluid hydrostatic pressure π_p and π_i coll idosmotic pressures of plasma and inter titial fluid) will increase net filtration (F) and IFV However several local mechanisms will tend to counteract edema formation and one may envisag three lines of defense (I III)

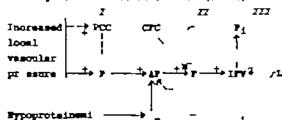


Fig 1. and Increase and decrease of indi cated parameter Broken arrows Edema-opposing mechanisms

I Through myogenic and/or local nervous eflex mechanism the ise in vascular pressure will induce precapillary constriction (PCC) and thereby educe effective capillary surface area and filtration coefficient (CFC) nd dimi ish the rise in P Both effects will oppose the ise in net capillary filtration (F)

II The second line of defense is activated by the increase of F and IFV A rise in capillary filtration and dilution of interstitial protein will reduce $_i$ and the increase of IFV will increase P_i ith resulting f ll in ΔP The relative magnitude of changes in P_i and $_i$ will depend on interstitial compliance A fall in $_i$ seems most important in skin muscle and inte tine

III Lymph flow (L) will increase in response to rise in P_i (and possibly IFV) Besides emoving water ise in L is necessary to maintain low $_i$ by removal of protein

Mechanism I is immediate and independent of changes in IFV and i probably the most impor tant in everyday orthostasis Mechanism II is lower, developing in the course f hours The part played by reduction in $_i$ provides only transient protection unless L i increased (III) Only mechanisms II and III ar activated in hypoproteinemia and possibly also by chronically levated venous pr ssure (g congestive heart failure) Tog ther II and III may oppose t least 10 mm Hg fall in $_p$ increase in P

With contraction f total ECV the same mechanisms tend to retain fluid in the interstitium Thus the plasma volume is not maintained unchanged t the expense f IFV Teleologically the resulting chang in plasma vol ume represent the necessary ignal to reflex control of enal water and salt excretion

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Wennergren G. and Oberg B (Departments of Physiology nd Medi trics University of Göteborg Göteborg and Department of Physiology College of Veterinary Medicine Uppsal Sweden) CIRCULATORY ADJUSTMENTS INDUCED FROM THE VENTRAL SURFACE OF THE CAT MEDULLA OBLONGATA.

Cardiovascular effects li ited by bil teral local cooling of and topical application of drugs on re- tricted area on the ventral surface of the medull ob- longata corresponding to the caudal part of the rostral chemosens t ve rea (Mitchell et l 1963) and the intermedi te area (Schläfke and Loeschcke 1967) have been tudied i chloralose-anesthetized cats (Wennergren and Oberg i pres)

Cool ng f or topical application of GABA or glycine on these tructures resulted in a asodil tation i the kidney and the skeletal muscles lowing of the heart, blood pressure f ll and lse depression of the respi ration Small rcul tory responses except for light increase i heart rate instead of lowing occurred with application of physostigmine

During application of the inhibitory transmitter substance GABA withdrawal of arterial baroreceptor restraint (carotid occlusion) produced only very small and markedly ttenuated increase in peripheral resi tance i the renal vascular bed whil carotid occlusion till led to substanti l vasoconstriction i the skel tal muscl vascul bed Arterial baroreceptor timul tion during topical GABA appl cation induced irtually no further vasodil tation the renal vascular bed whil learcut vasodilatation was still produced in the skel tal muscl vascul bed These findings suggest that GABA pplicat on leads to a general diminution and i the kidney irtually compl te cessation f the tonic vasoconstrictor fibre activity

Topi l application of methyl tropine itrate induced blood pressure ise and an augmentation of vasoconstri tor fibre tone i renal and kel tal muscl vascular beds After methyl tropine application the reflex vasoconstrictor response i the kidney to removal of arteri l baroreceptor restrai t was drastically potenti ted whil that in the skeletal muscl vascular bed was less affect ed

The tudied medullary tructures seem to pl y an important rol for the maintenance of the toni asocon trictor fibre activity especi lly that to the kidneys It seems as if the degree of engagement of the renal scula bed i the arteri l baroreceptor reflex is particularly sensitive to changes i influences from the tudied structures on the ventral medull whil the skeletal muscl vascular bed seems less ffected The lterations i the baroreceptor reflex response pattern obtained in these experiments upon drug application on these areas on the medulla surface losely resembl the lterations in baroreceptor reflex response pattern which are observed during hypo- and hypercapni respectively

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Wennergren G. and B Oberg Pflügers Arch 1979 I press

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Svensson, T H Engberg G and Thorén P (Departments of Pharmacology and Physiology University of Göteborg Sweden)

ACTIVITY OF BRAIN NORADRENERGIC NEURONS IN SPONTANEOUSLY HYPERTENSIVE RATS

Noradrenergi neurons in the pontine nucleus locus coeruleus (LC) hav been implicated i the mediation of arousal and anxiety reactions as well as in the regulation of autonomic g cardiovascular function. Thus electrical stimulation of the LC has been found to produce pressor response which however was reduced in spontaneously hypertensive rats (SHR). Moreover afferent stimulation of the vagus nerve was found to cause inhibition of LC neuronal acti ity We hav used singl cell recording techniques to further characteri   the LC function in chloral hydrate anaestheti ed SHR The average firing rate of randomly encountered LC noradrenergi neurons was progressively reduced with increased age and blood pressure of SHR when compared with normotensive agematched Wistar Kyoto control rats (WKR). Bilateral vagotomy in the neck did not affect the reduction in LC neuronal firing rate The activation of LC norepinephrine-containing neurons by yohimbine an α2-receptor antagoni   was more pronounced in SHR than i WKR suggesting deficient autoreceptor function i the LC of SHR These receptors seem to mediate noradrenergic autoinhibitory influenc from axon collaterals which can serve to dampen the impac of repetitive stimulation of LC neurons. In biochemical experiments the in ivo rate of tyrosine hydroxylation was found to be equal i LC neuronal activity of SHR is depressed below normal in anaesthesia in parallel with thei cardiac output and heart rate The NA neurons in the LC of SHR may consequently be particularly sensitive to environmental stimuli

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E i son, L (Department f Physi logy College I V t inary Medi ine Hel inki Fi land)

CENTRAL CONTROL OF BLOOD PRESSURE POSSIBLE ROLE OF HISTAMINE

The rol   f noradr ne gi and serotone gi sy tem in he ce   l con rol f blood pre ur ha been tho oughly tudied nd gene lly ccepted Also adrenergi   cholinergi and dopaminergi neuron   re known o participat i thi control but th i precise rol i not yet l

I   ecent ye   l rge body f evidence for po ibl   urotransmi t   function f hist mine ha   ccumul ted (f Schwa   Ann Rev Pharmacol Toxicol 1977 17 325) The uneven di t ibution f h tamine   he br in and it loc li tion i synapt somal f actions   well decr sed synthe i f llowing specif   lesion   d lec ophy   log   l evidence f vou the   i tence f hi taminergi pathway   The   na onl l imil it   be ween hi tami ct   nd no ad ene g   nd serotonergi sys em

Hi tamine gi en   lly ha been found t ffect veget ive functions nd beha i   Al ady i 1937 Trend lenburg (C rc Re   5 105) ob rved an lev ion f rt i l blood p ur   nd   in   se i he   f llowing   i j tion f h tamine in the hl i se e heti ed   Th   ffect wa probably medi ed by   es r l nc   ympa heti pre I mo   tudies   ypr   ive ffect of histamine could be blocked by hi   e ne H_1 eceptor   t goni   wh   he p   tmer with the selective H_2 eceptor an go   did no modify he r sponse   T   t ha been   ug ge ted that the   ffect   f   tam   p e- domina tly medi ed by H_1 ecep   imul ion

I ane theti ed animal hi tamine given centr lly con i tently increase the heart   te where   in conscious animal the he rt   t doe not change   decreases Bradyc rdia may be   baror fl   spons   yet the he t r t ha been eported to be low even ft r blood pre sur ha been normali ed (Hoffma & Schmid Lif Sci 1978 22 1709)

The avail bility of selective hi tamine H_1 and H_2-agoni t ha mad it po ibl t stimu l   these ecept   sepa t ly I   injection f H_2 agoni t dimaprit ha been hown o lev t blood pressur and he t rat i urethane-ane theti ed   t   nd these ffect   blocked by H_2 eceptor antagonist meti ide (Hick   Cli   Exp Hypertension 1978 1 251) Also in con iou goats i   inf ion f high doses of dimap it incr sed blood p   ur   but the latency ime   well   the dur tion f ction we   ften pr longed On the other hand H_1 agoni ts   pidly lev ted blood pre sur (Erik son & Tuomi t   t be publi hed)

The ite   f   rdiov scul   ction f hi ta in   re not defined be use i most tudi hi tamine ha been gi en i   By   ing micro-i jection r ponsive   hav been found i po   i   nd   t ior hypothalamu (Bre enoff   Jenden Int J Neuropharma   1969 8 593 Fi ch & Hi k   Ne opharmacol 1977 16 211) These howeve   nay not be the only r pon ive t

As ompa ed t the well nalysed di ect d ov cul   ffect   f hi amine i f rmation on   en   lly medi ted ction i me yer   wha   ten   hi blogy i amine p rti ipat i   he ce   l cont l f blood p   r ma   t be lucidated

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Dalbro D and Lisander B (Department of Physiology University of Göteborg, Sweden) HYPOTHALAMIC CARDIOVASCULAR AND GASTROINTESTINAL INFLUENCES

Adrenergic nerve control is partly organized at spinal level This is clearly the case concerning the sympathetic nerves to the gastrointestinal tract where motility and circulation can be decreased by activation of the spinal gastrointestino-gastrointestinal (GI-GI) reflex elicited by distension of the gastrointestinal tract or nociceptive stimuli within the abdominal cavity Experiments involving section of the spinal cord indicate that the GI-GI reflex is under net inhibitory supraspinal modulation whereas cardiovascular control is in general known to be under strong supraspinal influence (cf Furness and Costa 1974) In the present study, experiments were performed to elucidate the interrelation between cardiovascular and gastrointestinal sympathetic effects elicited from the hypothalamus and whether the GI-GI reflex can be influenced from this structure

The hypothalamus was stereotactically explored in chloralosed adrenalectomized cats The cut vagi were efferently stimulated to elicit a constant and submaximal tone in the stomach The volume of the latter was recorded with the balloon method together with blood pressure and heart rate

Hypothalamic depressor or bradycardic responses were paralleled by decreases in gastric volume However, pressor responses indicating an increased adrenergic tone on the cardiovascular system in general were likewise associated with gastric contraction whereas stimulation induced tachycardia was not clearly linked to any particular type of gastric response

Thus the adrenergic activity in the stomach and the cardiovascular system did not always change in parallel A possible explanation was that the activity of the GI-GI reflex and cardiovascular neurons in general were independently influenced from the hypothalamus As the animals were laparotomized a high degree of activity in the reflex could be supposed

In acute experiments on cats with a chronic Thiry-Vell loop, the GI-GI reflex could be elicited at will without prior laparotomy Distension of the innervated intestinal loop elicited marked and prompt increase in gastric volume Stimulation of the hypothalamic depressor area decreasing blood pressure and heart rate (Folkow, Johansson and Öberg 1959) abolished the gastric inhibition during the GI-GI reflex but did not influence gastric tone when the reflex was absent. It is concluded that the GI-GI reflex can be inhibited from the hypothalamus and this influence may be exerted independently of changes in cardiovascular neurons in general

References

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FOLKOW B , B JOHANSSON and B ÖBERG Acta physiol scand 1959 47 262 270

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Sundler F Alumets J , Håkanson R and Lorén, I (Department of Histology and Pharmacology University of Lund Lund Sweden): ABERRANT LOCALIZATION OF PEPTIDE HORMONES WITH SPECIAL REFERENCE TO THE ABDOMINAL ENDOCRINE ORGAN

The last decade has witnessed a virtual information explosion in the field of peptide hormones and neuropeptides This progress has resulted from the development of immunological techniques (radioimmunoassay and immunohistochemistry) Several recent studies have indicated the occurrence of peptide hormones and neuropeptides in unexpected locations Thus certain peptides of the adenohypophysis (GH prolactin ACTH α-MSH β-lipotropin and β-endorphin) and of the gut (gastrin CCK GLI and VIP) have been found to occur in the brain Conversely peptides first demonstrated in the brain (such as somatostatin neurotensin and the enkephalins) occur also in peripheral organs notably the gut and pancreas In the following examples are given of aberrant localization of still other hormonal and neuronal peptides

Certain growth hormone antisera demonstrate GH-like immunoreactivity in the antral gastrin cells and in a population of pancreatic islet cells Other GH antisera while being potent in demonstrating pituitary GH cells fail to demonstrate the cells in the periphery indicating that the immunoreactive material in these latter cells is distinct from pituitary GH.

ACTH-like immunoreactivity occurs in scattered endocrine cells of the gut and pancreas of certain mammals as well as in nerve fibers of the porcine gut Immunohistochemical analysis using several ACTH antisera directed against different regions of the ACTH molecule indicate that the immunoreactive material is distinct from pituitary ACTH CLIP (ACTH 17-39) and α-MSH (ACTH 1 13)

Antisera against GIP (gastric inhibitory peptide) have been found to demonstrate not only endocrine cells in the duodenojejunum but also numerous endocrine cells in the pancreas and large intestines These latter cells are identical with glucagon cells and GLI (glicentin) cells respectively Since some GIP antisera fail to demonstrate glucagon and GLI cells the immunoreactive material in these cells is probably distinct from duodenojejunal GIP

Certain motilin antisera are capable of reacting with the pituitary ACTH and MSH cells The immunoreactive peptide is chemically related to but distinct from authentic motilin Thus the ACTH and MSH cells contain a motilin-like peptide in addition to the endorphins and the polypeptides having NH_2-terminal tryptophan

Somatostatin immunoreactive cells are abundant in the pancreas and gastrointestinal tract In addition somatostatin immunoreactivity occurs in parafollicular cells of the mammalian thyroid and in the avian thymus In the thyroid the somatostatin cells contain immunoreactive calcitonin as well In addition there is a population of calcitonin cells which does not contain somatostatin The granules of the calcitonin cells are morphologically distinct from those of the somatostatin cells

Several of the observations above concern tissue components which resemble known peptide hormones without being identical Although they are recognized by antibodies against the peptide hormone in question they do not possess the exact immunological properties of the full peptide This may be explained in one of the following ways:

1 Immunological cross reactivity The immunoreaction may reflect the presence of chemically related but physiologically unrelated peptides

2 Peptide fragments. The immunoreaction may reflect the presence of fragments (bioactive?) of the peptide rather than the peptide itself

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Lundberg, J.M. (Dept of Histology Karolinska Institute Stockholm Sweden) ENKEPHALIN SUBSTANCE P VIP SOMATOSTATIN GASTRIN/CCK AND NEUROTENSIN IN PERIPHERAL NEURONS

The presence of enkephalin(ENK) substance P (SP) VIP somatostatin (SOM) gastrin/CCK (GAS) and neurotensin (NT) like immunoreact vities in the peripheral sympatheti (S) and parasympathetic (PS) nervous system of the cat was studied using immunohistochemistry Adrenergi systems were visualized using antibodies to the catecholamine (CA) synthesizing enzymes and holinergi neurons with acetylcholinesterase staining (AChE) Normal cat tissues and ganglia as well as specimens from animal subjected to nerve ligations denervation and local vinblastine applications were investigated It was found that all peptides studied (ENK SP VIP SOM GAS and NT) were axonally transported mainly in ant rograde direc ions The following general dist bution patt rn and neuronal projections were found ENK was observed) in presumably preganglionic axon leaving the CNS vi 1) the vagus and pelvic nerves (sacral ventral spinal roots terminating in PS gangli of e g re piratory and urogenital tracts and in 2) thoraci ven ral spinal root extending into e g splanchnic nerves terminating in S ganglia b) in postganglioni PS neurons in ganglia innerva ing exocrine glands e g phenopala ine and pancreatic ganglia with terminals in the parenchyma; and in Auerbach' plexus of the gut innerva ing smooth muscle, particularly in sphincter regions and blood vessel SP was present) in sensory neurons of e g nodose and pinal ganglia projecting with peripheral branch into e g vagus planchnic pelvic and sc atic nerves to various areas including respiratory tract heart gut S and PS ganglia urogenital trac and skin (innervating ganglion cells and blood vessels as well as be ing free nerve endings, and b) in postganglionic PS neurons of the gut innervating smooth muscl VIP was observed in) postganglionic PS neurons of the respiratory and urogenital tract heart (few) pancreas gut (both in Mei ssners and Auerbach lexa) sphenopalatine otic submandibular and pelvic ganglia innervating 1) blood vessels and smooth muscl of the gut respiratory and urogenital tract particularly smooth muscle of sphincter regions e g pylorus and cervix uteri and 2) exocrine glands e g pancreas nasal lacrimal, submandibular bronchial anal and uterine endometrial glands Nerves stained for VIP and AChE were ften closely overlapping in exocrine glands of e g nasal mucosa. AChE-rich and VIP positive cell of e g sphenopalatine and pelvic ganglia were often dentical suggesting coexistence of VIP and acetylcholine b) postganglionic S neurons (mainly tellate and L7 S1 ganglia) projecting via sciatic nerve to sweat glands and some skeletal muscl resi tance vessels AChE ri h and VIP cells were losely overlapping also in these ganglia indic ting further presence of VIP in cholinergic neurons) some VIP gut neurons project back to prevertebral gangli (e g coeliac and mes inf ganglia) Mesenteric nerve axons however do not seem to be AChE-rich and d) sensory neurons f nodose and pinal ganglia with unknown destination However some free nerve endings containing VIP have been observed in the urogenital tract as well as many nerve endings in the carotid body SOM was present in) sensory neurons of the nodose and spinal ganglia the latter possibly projecting to S gangli b) postganglionic PS neurons of the gut (mainly in Meissners plexus of intestine) extending into the villi GAS was found in) sensory neurons of the nodose and spinal ganglia. Peripheral axons in e g pelvic and sciati nerves and partly around blood vessels and partly as free nerve endings b) postganglionic gut neurons (mainly distal intestine) and) neurons projecting from gut to prevertebral ganglia (see VIP) NT was mainly observed as preganglionic axons projecting via S pathways (thoracic ventral pinal roots) into e.g splanchnic nerves terminating in various S ganglia CA containing chromaffin ell of the carotid body contained ENK; SIF-cells ENK and SOM; adrenal medullary cells ENK and NT respectively

Fahrenkrug, J (Department of Clinical Chemi try Bispebj rg Hospital Copenhagen Denmark) VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) A PUTATIVE NEUROTRANSMITTER

V soactive intestinal polypeptide (VIP) is highly basi octacosapeptide riginally isolated from porcine small intestine in 1970 VIP has wide range of bi logical ffects among these vasodilation laxation f non-vascular smooth muscles and timulation of pancreatic bicarbonate secretion

VIP was originally considered to be gut hormone but recent radioimmunochemical and immunohistochemical tudies have revealed that the peptide has wide-spread distribution in the body localized i neurones In the ce tral nervous system VIP ergi nerve terminal and cell bodies are primarily located to the cortex hippocampu hypothalamus and amygdal id complex In the digestive nd genitourinary tracts VIP rgic nerve fib es of beaded appearance innervate small blood vess ls smooth muscl and epithelial cells

VIP is localised in ve icles within the nerve terminal from which it can be released by depolarising timuli Whilst the physiological role f VIP in the central nervous system i still unknown evidence has been provided that VIP i the neurotransmitte f the f llowing gastrointestinal and pancreatic functions known to be mediated by non-cholinergic non adrenergic autonomic nerves fib es 1) the gastric receptive relaxati n 2) the testinal hyperaemia li ited by mechanical mu sal ti mul tion; 3) the vasodilation f the colon provoked by ctivation of the pelvi nerves; 4) the vagally induced pancreatic bicarbonat secretion

Acknowledgements Some of the studies wer performed in collabo tion with Drs U Haglund J J Holst M Jodal O Lundgren L Olbe and O B Schaffalitzky de Muckadell

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Uvnäs-Wallensten K, Efendić S, Järhult J, Lundberg J.M. (Departments of Pharmacology and Histology Karolinska Institute and Department of Endocrinology Karolinska Hospital Stockholm and Department of Surgery University Hospital Lund) RELEASE OF PEPTIDES FROM ENDOCRINE CELLS AND NERVES

The l of the tudy was t tudy mechanisms which con rol the release of peptides from endocrine cell of the gastrointes inal t act and from pe ipheral nerves

The release of gastroi es inal hormones was udied i acute expe iment i cats The portal vei was cannu ted and portal blood concen ations of gas i gluka gon insuli and somatostati were measured The ca were lso provided ith acute an ral pouches which were perfused with 0 1 M HCl or 0 15 M NaCl Since gas rointestinal hormone re released both i the ircul ion and i to the gas rointes inal lumen gast i and somato ta i level of the pe fuse as we lso determined

Elec ical vagal timulation increased the release f gas i and somatostati i to the perfuse and increased the ou pu of gast i insuli and glukagon i to he portal vei whereas port l somatosta i level decreased

The i raluminal secretion f soma osta i was enhanced only when vagal imul ion occurred at acid i antral pH whereas gas ri release was considerably highe antral han at low pH f he perfuse Si l ly activa ion f the sympathe i nervous ystem or infusion f adrenaline caused ligh increase f basal gas i level new ral i an ral pH whereas somatostati was released acid pH

The peptides f the endocrine cell f he GI t act re mos probably leased not only i to he blood ream but lso into the intera i i i fl id surrounding the cell Therefore t low i ream l pH the local concen ion of somatost ti ough to be high around he G cell Since vagally induced gas i release can be blocked by i fusion f somatosta i (0 5 µg/kg/mi) we ugge that the inhibitory ffect on gas i release observed at low i traantral pH i medi ted i direct ffec of somatost i on the G-cell

Vagally induced gas i release was reduced to 30% of con rol lues when imultaneous ac ivation of the sympathe i nervous system was performed or by i fusion of NA (5 µg/kg/mi) Thi inhibitory mechanism was compl tely independent f intraant l pH We think tha thi inhibi ion i caused by an α-adrenergi inhibition of the vagal impulses which igh be exerted the ganglional level

T lbutamide or glibenclamide given i or i the an ral perfuse caused an increase of gas i and somatosta i level i the lumen and release f gast i in the blood tream. Obviously the secretion of gastro i tes inal hormones can be imulated by subs ance applied ithe i the ircul ion or i the lumen

Due the unique morphological properties of the endo ine cell of the gas rol estinal t act the rate f gas i release i con inuously under eral factor i con rol Thus the imul tory vagal tone can be coun r ac ed by ac ivation of the sympathe i nervous system but lso by loc l factors i the antrum such as low intraantral pH f rthe more different chemical compounds can i fluence he rate of gas i release both i the lumen and i the ircul tion

When the sci ti or brachi l nerves of extirpa ed cat legs which were perfused ith Tyrodes sol ion were ti mulat d lec ically gas i 17 and an insulin-like substance appeared i the perfusate Simil ly the iferent drugs tolbutamide and glibenclamide added the perfusate caused an increase of gas i and the insulin-like mate i l i the perfusate

At present i can not be defi itely tated whethe these peptides re released from nerve endings or from some othe l use depot Howeve if the gas i and the insulin-like immunoreacti ity iginates from nerve fibre triking imilarities exi between the ways by which pep ides can be released from endocrine cell and nerve

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Efendić, S (Department of Endocrinology, Karolinska Hospital Stockholm Sweden) SOMATOSTATIN A HORMONE OF THE PANCREATIC D-CELLS

Isolation and synthesis f somatostatin was soon f llowed by the finding that the peptide inhibit the secretion of number f periphe l hormone Shortly af erward we demonstr ted that soma ostatin is also pre ent outside the hypothalamus in ther part f the nervous system as well as in everal peripheral endocrine lik cell g the D-cell f the pancreati islets

We have inve tigat d the possible physi logical ignificance f somatostatin for the regula ion f the functions f the endocrine pancreas by two pproaches firstly by tud ing the ff ct f exogenous omatost in an secondly, by trying t elucidat mechanisms controlling the release f the peptide

Exogenou somatost i decreases basal and timula ed insulin l vels in vivo and suppre ses he insulinogeni ff f glucose, argi nine olbutamide and glucagon S milarly omatost tin inhibi ba al and timulated glucagon release

Somatost tin rel ase as tud ed in the perfused rat pancre as well as vo l rats cats and dog The peptid measured by RIA using own an ibodi Glucose arginine and sulphonylureas ignif cantly timula d omatoti release El ctr cal timul tion of the agal nerv resulted in decrease in portal vei omatosta in The and ther tudie g hat soma os in may play an important rol as loc l paracrine regula or f the re l se f insuli and glucagon

As f he mode f ction f somatosta in lable da sugg t tha in erf rence with the cellular level f cyclic nucleotide may be of ignificance

The clinical use of somatostatin has been xplored especially in diseases characterized by hypersecretion of hormone known t be in hibited by somatostatin howeve , the ide distribution and multipotent inhibitory action f the peptide calls for ttention Analogues to somatos tin ith pecifi tions have al ready been produced and await linical evalua tion

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Ml p, K (Dep rtme t f Phy i l gy U i f T k d Ku pi T ku and K pi Fi l d)

BIOLOGICAL SIGNIFICANCE OF EXTRAHEPATIC DRUG BIOTRANSFORMATION OPENING REMARKS

Th li h g lly b kno l dg d h j it f h drug h li E tly i i g i h h p id th bi f rma i ti i h h p i i he th fi it p lly dmi i d drug d th bi ti me l t Th body h l f en time oupe d by t f rei g hem i mabbl t bolit d by i g th [illegible]

Th i i fi di g i hli hi g h i t f t h p i d g m bolism h h h d i f h gl id i mat i i he g i t i i (Ha i l K A Med E p F 33 239 1955; Hem H p Pari 32 1 1956) La h i i l me h be fou d t t ib ly ll yp f d g h li i g i j th i t tinal mi fl y ev p f rm h i h li i hi p (Ha i l K Physi l R 53 456 1973) Whil h lyti l hed h p g d mam en h p i l ha b f d id d j g f ig oup d (V i l K d Hi K I C p i Drug Me bolism Ed B T d P J an Ma l D hh I p 1979) It igi lly hought ha drug me b li i g en yme y em ill ly d ify mobi i j l t ly l i i h he f d h pl K h gh rvi ma t l mobi h b dy l d h y ill p hl t p d d id ff by f rm g i i reedi bl d wi h me onol ual

Th ti en (g i d l g) hi b the pe f ry f mobi i hav f en qui high bl t f reacti ti i y ith i d d by l d pe t hi l d g ly Th h p i drug t bol i ing ti i y i highly iti the i me l t l Th i t ti l i i y h modifi d by he iti mal f (Hie ea K d La g M I Conj g tion React i D g Bi f rm i Ed A Al i El e i /N h Holl d p 199 1978) Th p p i f food y i trad pred i g yf ld i d i i h i t ti l drug me h lism in th way tha h hl ed ea tr ti f p lly dmini t d drug be l d (C y A H l F d P oc 36 1647 1977)

P ibly the l f ex h p i drug me b lism ha be l l d f om h poi t f ph rma ki ti and l he ig ifi an f ext h p i ti ha h gl d i h rm Howe d ing h emp l dmi i tion h drug h lung pa th ki lung g i inal me whi h ti igh i t p i ipa i he fi p ff hef h drug t lly h p l sy tem d pa he li (Ha dy K G nd Gill J R J Ph rma ki Bi ph rmace 6 355 1978) I th ind d h h p i drug bolism may l p i ipa i drug ion I h i ti l well hemi als y be t bolit d thei j g t nd ex d h k h i i l lue wi here ev ing h y em ire l i (J ting B l Biochem Ph rma l 25 613 1976; Hi K t l Ab t XI I Cong f Biochem I p 1979)

O ht Th igi l h by th the we pp t d by h NIH G t(R01 ES 0168) nd J V i i y d i

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Orrenius S , Graf tröm R , Ormstad, K, and Moldéus , P (Department of Forensi Medicine Karolinska Institutet S 104 01 Stockholm Sweden) ISOLATED CELLS FROM EXTRAHEPATIC ORGANS AS A MODEL TO STUDY DRUG BIOTRANSFORMATION

Intact isol ted cell from rat kidney and small intestine were prepared by enzyme diges tion kidney cell using recirculating perfu ion system containing collagenase intestinal cells by incubation of the intestinal mucosa with collagenase and hyaluronidase

After cell preparation 85 95% f the cells were viabl as judged by trypan blue and NADH exol ion No loss f iability was observed within 2 3 hours

Both cell types catalyzed cytochrome P-450 dependent oxidation reactions as well as conjug tion with glucuroni acid sulphat and glu tathione These reactions proceeded t maximal rates indicating sufficient endogenous production f cofactors Induction f cytochrome P-450 oxidation by 3-methylcholanthrene was observed using paracetamol harmine and benz ( ) pyrene as substrates The effect was most pronounced for *benzo( )pyrene oxidation* in the intestinal cell where a 10-f ld timulation we achieved

Of glucuronidation and sulphatation glucu ronidation had highest ctivity in both the kidney and the intestinal cell In kidney cells sulphatation was about half of glucuronidation whereas only trace amounts f sulphate conjug tes were f rmed in the intestinal cell The activity f these two conjugation reactions as well as that f the cytochrome P-450 dependent oxidation we considerably less than in isol ted hepatocytes Thi was true also f gluta thione conjugat formation However the con ver ion f thi conjugat to the cyst ine and N-acetylcysteine conjugate reactions absent in isolated hepatocytes was pid in both kidney and intestinal cells Of the two cell types kidney cell had the highest activity

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Ootil P Toivonen, M Simberg, M Wienstedt, W and Marti la J (Department f Physiology University f Turku Finland) PULMONARY BIOTRANSFORMATION IN DRUG EXPOSURE

Many environmental compound may ente the body via the lungs eg in polluted ity ir and cigarette smoke These compounds in addition to endogenous steroids and certain asoactive hormones are metabolized in the lung

Exposure f rats to igarette smoke increased the metabolism f benzo( )pyrene carcinogenic compound present i cigarette smoke in pulmonary microsomes (Ootila et al Cance Res 37 2136 1977) and in isolated perfused rat lung (Cohen et al Cancer Res 37 2147 1977) Cigarette smoke increased the metabolism and covalent binding of benzo( )pyrene also in the trachea f the rat The rate f metabolism in the trachea was returned to the control level soon as in 24 hrs (Simberg and Ootila Int J Cancer 22 28 1978) Although cigar tte smoke exposure increases the pulmonary metabolism of benzo( )pyrene and thus the formation f active intermediates several f ld the te f formation f detoxified water soluble conjug tes is rather small (Ootila In Conjugation Reactions in Drug Biotransf rmation ed A.Aitio Elsevier/N-H 1978 29) This may be one mechanism for the initiation of lung cancer.

Styrene i an industrial hygieni problem in plasti industry Inhalation f tyrene increased the activities f drug metaboli ing enzymes in rat liver but not in the lung (Sandell et al. Res Commun Chem.P thol Pharmacol 19 109 1978) After intratracheal instillation into isolated perfused rat lung styrene oxide the primary oxidative metabolite f tyrene wa absorbed rapidly into the perfusion medium (Ootila Res Commun Chem P thol Pharmacol in press) These tudies indicate the envir men al xenobiotics may be absorbed through the lung into the body concentrations which may have harmful consequences

Many teroids such as testosterone (Martiala, J t al.,J Steroid Biochem 7 527 976) and progesterone (Martiala,J t al J Steroid Biochem in pres) are metabolized in the pulmonary circulation The rate f pulmonary testosterone metabolism seems to be controlled rather ffectively as the rate of formation f hydroxylated polar metabolites f testosterone was increased only lightly after cigarette smok exposure (Martiala J et al J Steroid Biochem 9 365 1978). Syntheti beclomethasone dipropionate used i th treatment of bronchial asthma was absorbed slowly into the perfusion medium afte intra tracheal instillation into isolated rat lungs Howeve the rate f its metabolism in the lung tissue was considerabl (Martiala,J Br J.Pharmacol 57 442P 1976) These results may account for the insignifi ant adrenal suppression observed in beclomethasone aerosol therapy

Smokers usually have lightly lower blood pressure than nonsmokers (Karvonen et al. Lancet i 492 1959) wherea those who top smoking have tendency f rising blood pressure (Seltzer,Am Heart J 87 558 1974) Cigarette smoke is able to relax the smooth muscl of isolated superfused rat colon This relaxation i not reversed by potent constrictors eg angi tensin II (Ootila and Toivonen unpublished result) One day fter the exposure f rats to cigarette smoke for 1 hr the conversion f angiotensin I to angiotensin II in the pulmonary irculation was increased Howeve this increase was not seen after 10 days' consecutive exposure (Nakhla t al B .J Pharmacol., 65 495 1978) This indicates that smoking may have significant ffect on blood pressure by ffecting the level f ircul ting hormone

Grants NIH (USA) ES-01684 18 Juho Vaini Found.

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P lkonen, O Ho l nen, M L, Rauni H, Ke i H and Ka ki N T (Dep tme t f Pharmac l gy U i rsi y f Oul Fi land) SIGNIFICANCE OF PLACENTAL DRUG METABOLISM IN FETAL DRUG EXPOSURE

Only negligibl xeb -met bol ing ti it ha bee demonstra ed in pl centas f om on smoking moth rs Of abou 30 substrates twd d in itro only 2 3 h been unequ ocally how t made g b ransforms on t any ten Th nduc b l y f he met bolism f ome sub t (b nz ( )py ene 7-ethoxy ouma 2 5-d phenyl xa l la lamine dye K methylan l) f smok ng mo h rs h bee l tabl shed Conj g t on t ons re ve y d f ent g d l f mat rnal b xpos Th i duc en f benz ( )pyrene hydroxyl on pl ent by ma rnal g re smok g fi t ppe th l eak f h f mest f p gn ncy and ns t h ghe t lev l t t rm Of he well known induc ph nob b l l ohol g t mothe during ly p gnancy do indu pl ent l me bol sm

The pl en l monooxyg nase y tem ha bee cha t t d by dy g ub pec f y p f l h b d SDS gel lectrophor l l ly abl bed ha he plac l) em enbl l ly h poly y l oma hyd oc bon ype if ytochrome P 450 l re re ome h spect t the t i ed sub t pe f ty (g ld i ouma in h ph nyl re met bol zed, whereas ben ( )py ene 2 5-d ph nyl l nd 7-ethoxycouma l met boli ed parall l manner, the pr f en l ah b t on by 7 8 benz flavone nd h p rn f prot i band th 50 000 d l on reg on f SDS-g l lect phore

Thus the pl t ca me boli only very li ted numbe f xobi ti th ough limit d numbe f pathway and thi fi ding may be xplicabl t rm f h gh sub trat specifi i i f the plac t l nzym component i ont t t the low sub tra specif f the rre ponding live ystems On th ba i on ma onclude that pl ental en yme systems d not on ti t b l t major ty f f g ompounds A pos ibl eption may be th inducibl ystem f poly ycli romati hydroc bons becaus it h bee demonstrat d th t mat rn l lg t smoki g ha an induci g ffect on mat rnal lymphocytes bu not on f l lymphocyt Th re on f this diff nc might be that the pl t l yme metaboli polycy li hyd rbon indu re od p event them ent i g t th f tus

Although pl t l yme d t seem t be mporta t preventing the po ure f th f tus t f c hemical h y may p oduc harmf l metab lit du g th metaboli f g p lycycl romati hydroc rbons I y tro plac t l ben ( )pyr ne hyd xyl se p oduc met b lit wh h re me g l and bl t b nd t DNA These tive l rmedi t may bind pl ent l mac omol cul t t f t d harmf l ffects As t th mat f he ffect only pecul ti an be p ted be va i bl ma ifest ion f f i lity, t aspl l mu gene i nd rcinogene i re pe ibl

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Vainio, H. (Depa tment f Industri l Hygiene and Toxicology Institute of Occupational Health Haartmaninkatu 1 H lsinki 29 Finland)
EXTRAHEPATIC BIOTRANSFORMATION IN DRUG INDUCED TOXICITY

The enigma of how inert chemicals can exert various adve se biological ffects including geneti and toxi damag and carcinogens is has been much debated It has been learned that such compound ar metabolically converted to chemically reactive pecies Thus the locali tion f enzyme which genar te these ea tive metabolite play an important rol in initi t ing the toxicity dev lopment For many of the hemical which equire metaboli acti tion to exert thei toxicity (g arcinogeni ity) the its f action appear t be determined prima ily by the final concentr tions f e active int rmedi t av ilabl f reaction with all lar macromolecules which esults f om ritical balance between acti tion and detoxication f th hemic l in vivo

Hence the i teraction f environmental toxins, carcinogen or mutagens with the gonads may alter the eprodncti capability by ffects on the gonadal tromal cell by ffects on the germ cell The results f uch inte tions may be impaired fertility gonadal tumor or germ cell mutations

Degen tion f nervous system in mammal in long-term expos to neurotoxi chemicals ppears to be t least partly mediated i the f rmation of reactive intermediates The brain tissue contains enzymes which are known to be abl t catalyz the metaboli cti tion re tions and whi h can explai the ppearance f metabolite neurotoxi ity f number f solvents (g n-hexane ca bon disulfide)

Recent evidence also suggests that environmental mutagen and their metabolism within smooth muscl cell in the rtery wall might be involved potential ti logi facto in the genesis of cardiov scular diseases The bi transformation of promutagens into active metabolites would then initi te the formation f monoclonal therosclar ti plaques

Lenticular opacification or cataract may be induced also by chemical and drug g napthalene and paracetamol Reacti intermedi tes are bound cov lently in the lens f g mice producing i revez ibil opacity in the len These ophtalmologi finding may be impo tant lso linically with egard to g occupational exposu to organi sol ents Among car pai ter len changes (nuclear aol roal co tical and subcortical opacities) have occurred with increased f equency as compared to ilway engines

Only few exampl hav been given here t indicate the ignificance f the extrahepati biotransformation in drug induced toxi ity Although quite larg perspectives exist it is still too early t predict its ultimate importance

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Guenthner Thoma H, Nena d, Raymond N nd Nebert Daniel W (National Institute f Child Health and Human Development and National Heart, Lung and Blood I titute National Institutes of Health Bethesda Maryland USA)
STUDIES ON THE CONTROL OF ARYL HYDROCARBON HYDROXYLASE IN RAT ADRENALS INDUCTION BY ACTH BUT NOT BY POLYCYCLIC HYDROCARBONS

Aryl hydrocarbon hydroxylase (AHH) activity i the rat adrenal cortex is specifically located in the microsomal f action 95 percent f the total AHH acti ity i microsomal while only 10 pe cent f the total P-450 content i microsomal Thi AHH acti ity an be distinguished from the majo P-450-dependent adrenal microsomal steroid hydroxyl se progesterone 21-oxida se ince the rmer i trongly inhibited by -naphthoflavone but the l tte i not Hypophysectomy decreases adrenal microsomal AHH ac ti ity to 6 percen f its former level progesterone 21 hydroxylase level decrea to 35 pe ce t f control values An electrophor ti band f 57 000 daltons which migrates identi cally t the band corresponding t AHH activity from r t live microsomes is also markedly reduced by hypophysectomy Exogenou ACTH treatment esto es both AHH acti ity nd thi lec tropho eti band to normal level However AHH acti ity i not induced i ither the intact hypophysectomi ed t by high doses f 3-methyl chol threne 2 3 7 8-tetrachloro-p-dibenzodioxin

These data show that AHH and proge teron 21 hydroxyl se are di inct enzyme acti ities and ar likely socia ed wi h diff ent f rms f adrenal microsomal cytochrome P-450 Adrenal i rosomal AHH ha properties very imilar to li er microsomal AHH (i i sen itivity to naphthofl vone inhibi ion and i presumed association ith the 57 000 dalton lectrophoretic band) but their sen iti ities to inducers are distinctly different That adrenal microsomal AHH is inducibl by ACTH and not by polycyclic hydrocarbons suggests that the mechanism f induction f thi enzyme i quit different from that postul ted f liver AHH eve though the enzymes themselves are quit imil

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Døving, K. B. (Institute of Zoophysiology University of Oslo Norway) TRANSDUCTION MECHANISMS IN THE VERTEBRATE OLFACTORY RECEPTORS.

The mammalian olfactory receptors have long cilia-like protrusions that are embedded in the mucus of the sensory epithelium. Each cell has about 17 cilia. The inner filaments have the usual 9+2 structure but lack dynein arms. Presence of cilia on the olfactory receptors of amphibi has been shown to be essential for the development of the electro-olfactogram, EOG. Some species of lower vertebrates have two types of receptors with cilia or with microvilli. The membrane of the receptor cilia bears intramembranous particles IMPs at density 19 times higher than that of the neighbouring cilia of the respiratory epithelium. These particles have been suggested to contain the acceptor structures recognizing the odorants. In frog there is positive correlation between the density of IMPs and the amplitude of the EOG to standardized olfactory stimuli. From the dog olfactory epithelium, protein fraction with specific binding to anisole has been extracted by affinity chromatography. Antibodies to this protein fraction applied topically to the olfactory epithelium of mice decreases the EOGs to anisol and other odorants.

Recording of the electrical activity of the frog receptors show that most cells have low spontaneous activity. Most of the stimuli that influences the activity of the cell tend to increase the spike discharge only few will suppress the discharge. Increasing the concentration increases the probability that the cell will respond to particular stimulus. Only tenfold increase in concentration is needed to reach maximum discharge frequency. A single receptor can respond to one or several groups of odorant. The evidence for the spatial concept of quality discrimination in the olfactory system will be discussed.

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Järvilehto, T (Department of Psychology University of Helsinki Finland) PROPERTIES OF MECHANORECEPTIVE FIBERS SUPPLYING THE HAIRY SKIN OF THE HUMAN HAND

The method of microelectrode recording from the human peripheral nerves (Vallbo Å and Hagbarth K.-E. Exp Neurol 1968 21, 270) enables detailed analysis of the types and properties of single peripheral nerve fibers in man. At the present the main knowledge on the transducing properties of the mechanoreceptors is based on electrophysiological work in different animal species. The present paper describes properties of mechanoreceptive fibers supplying the hairy skin of the back of the human hand.

The fiber sample consists of 258 single fibers in the superficial radial nerve. On the basis of the responses to static pressure 83 fibers could be classified as rapidly adapting (RA) fibers and 175 as slowly adapting (SA) fibers. On the basis of receptive field characteristics and responses to stretching the skin 33 SAI and 69 SAII fibers were identified.

The receptive fields of the fibers ranged from spot-like fields (below 1 mm in diameter) to large fields of several cm². Some fibers responded exclusively to stimuli moving over the receptive field. Conduction velocities of the fibers ranged from 8 to 90 m/s the mean being 54.7 m/s. There were no significant differencies between the conduction velocities of the different fiber groups.

The static discharge of SA fibers was studied by interval analysis during continuous pressure exerted by Frey hairs or by the tip of an electromechanical vibrator (indentation of 1 mm). The static discharge of SAI fibers was usually quite variable the standard deviation of the interval histogram being in most cases over 50% of the mean interval (coefficient of variation, CV). In contrast, most SAII fibers had an extremely regular static discharge (CV below 10%). 30 SAII fibers had resting discharge of 3.6 – 14.8 imp/s.

The dynamic discharge of the fibers was studied when using tactile pulses applied to the receptive field by the tip of the vibrator. Pulse amplitude was varied between 50-950 µm and pulse frequency was 20, 60, or 150 Hz (one cycle sine-wave). The latency of the first impulse in the response was dependent on the stimulus amplitude being reduced by 5 ms from the lowest to the highest amplitude on the average.

Stimulus-response functions of the fibers were calculated when using three different response measures: 1) the number of impulses 2) the first interval, and 3) the mean interval. The fibers responded to the pulse indentations with few impulses maximally with 7. It was typical of the fibers that they usually had an optimal pulse frequency at which they elicited the maximal number of impulses. When the number of impulses was used as response measure the stimulus-response relation could in most cases be described by power functions with exponents below 1.00 over the whole frequency range. The first interval or the mean interval in the response did not have an unequivocal relation to the stimulus amplitude. The data indicate that the amplitude of pulse indentations is coded by the number of impulses both in SA and RA fibers, whereas coding of the pulse velocity is based on the differential sensitivity of the fibers to pulses of varying velocity.

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Sand, O (Dept Physi l Veterinary College of Norway Osl Norway) THE ROLE OF Ca^{2+} IN THE HAIR CELL TRANSDUCTION PROCESS

Rece t intracellular recordings from hair cells i wide range of acoustico-lateral rg-ans how eceptor potential i response to me-chanical timuli In the mammalian cochlea and vestibular system the hai cell face an endo-lymph with potential exceeding +120 mV and wher K (150 mM) is the domina t cation The driving f rce for K^+ i inward through the pex and outward through the cell body suggesting that K^+ might carry the eceptor current Cupu-lae of the amphibi lateral line are exposed to f esh wate wher the dominant cation is Ca^2 The mechanosensitivity f the organ depe-nds upon the Ca^2 concent tion i the medium and is blocked by Ca^2 competitors (Sand J comp Physi l 1975 102 27) indicating that Ca^{2+} rries th receptor current Howeve ion selective lectrodes revealed K^+ concentr tions up to 100 mM and corresponding potential up to 50 mV in lateral line cupulae (Russell & Selli ck J Phy i l 1976 257 245) An lectrogen-i K pump wa postulated and it was uggested that K^+ carri the receptor current In high Ca^2 low K^+ sol tion amphibia hair cells re lectrically xcitabl (Hudspeth & Corey Proc natn Acad Sci USA 1977 74 2407) The le-trosensory cells in the organ f Loren i i which are embryologically rel ted to hai cell nd f os Ca^{2+} ich canal fluid gener te Ca^{2+} pikes in the presence f TEA (Clusin & Bennet Biol Bull ma bi l Lab Woods Hol 1973 145 429) Ca^2 i pas ively di tributed i am-phibian lateral line cupulae whi h leads to low Ca^{2+} concentrations f 10^{-5}-10^{-4} M due to the positive endocupula potential (McGlone t l J exp Bi l 1979 I press) The inward driving f rce for Ca^2 is not increased by the lectrogeni K^+ pump inc the positive endocu-polar potential tends to exclude Ca^2 from the cupula It is therefore lik ly that K^+ i the main arrier f eceptor current in these orga-ns even if the low i tracellular concentr tion of Ca^2 till causes an inward driving force f thi ion Similar arguments are pplicabl for the cochlea whe the Ca^2 concentration in the endolymph is $3x10^{-5}$ M (Bosher & Warren Nature 1978 273 377) The endolymphatic equi lib ium concentr tion rel tive to the pe ilymph is $7x10^{-7}$ M indicating that Ca^2 is actively transported It i pos ibl that Ca^2 control the membrane permeability for K^+ (Heech & Stan-den J Physi l 1975 249 211 Clusin t l Natur 1975 256 425) Ototoxic antibi tics di place Ca^2 from polyphosphoinositides if in-ner ea tissue (Schacht J Acoust Soc Am 1976 59 940) Thes lipids ar believed t ct in the contr l f membrane permeability Flock t al (Acta Otolaryngol 1977 83 85) have ecently shown that the hai cell cilia act as tiff rods pivoting around their base where maximal memb ane distortion will occur The tereocili contain highly rganized actin filament A imilar actin pattern i seen in the sperm acrosomal process Formation f this tiff protrusion starts when the sperm contacts the Ca^2 rich egg jelly It is tempting to pe-cul te that Ca^{2+} influence the mechanical pro-perties f the ensory hairs and thus the cou-pling between the transduce membrane and the mechanical timulus I conclusion The mechan-ism f hair cell transduction is unclea Ca^2 seems to have complex rol possibly acting t sever l different levels

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smila S, and Reute , T (Department f Zoology Un ver ity f Hel inki Fi land) LONGITUDINAL SPREAD OF ADAPTATION IN THE VERTEBRATE ROD OUTER SEGMENT

Photoi omerization f rhodop in i the di membrane f the rod u itiat pro es whi h i crease decreases the amount f ignal carri molec l (Ca GMP ?) in the yto-plasm Thi change in concentration f the ig nal carrier use losing f odium hannel leading to hyperpolarizat on f the ll The delayed time ourse f phot re pons indi t sev ral es lve ampl fi tion processe (re-tions) bef re the channel is losed Both ba kground light and an exposure bleaching ral per cents f rhodopsin bring bout dis-pla ement f the timulus-re pons urve pre-dominantly t the direction f large stimulus flash intensiti s Two diff rent type f mech-anisms causing thi di placement may be ug-ge ted 1) l al daptation meaning that the photoprodu t brought about by the adapting light aff t only their own di g by de-cre ing there the release f the gnal carri and 2) spreading adapt on meaning that de ensitizati pread from the bl hed di ou ward pos ibly int the whol rod Fo xampl the photoprod ct could dire tly af fect the amplif ca ion re tions i the yto-plasm or they ould produ desens ti ing agent whi h d ffuse to othe di t the out membrane

W have tudi d the preading f adapt tion by comparing th ffect f two kinds f bl ching and timulating light firstly light whi h i preferentially bsorbed by the di tal end f the rod and ondly light the bsorption f whi h is nearly homog neou ly dist ibut d in th rod The inhomogeneous b rption wa hi ed by using an blique blue-green light beam hitting the receptor ide f the fl t-mounted retina t an angl f 10° To furthe f our di tal absorption thi light wa polari ed with it lect i vector in the plane f the di membranes Thu 70% f the in id t light wa ab rbed in the di tal one-thirds f the rods

Ble hing everal pe cent f rhodopsin in an isolated frog retina lead t larger tran-i t red ct on f ensiti ity (i termedi te adaptation) and t small permanent decreas f sens t ity The permanent displacements f the timul re pon curve to th right (log-log plot) aft 10% bl aches were as follow (log unit)

Stimulu	Inhomog	Homog
Inhomog bl h	0 79	0 54
Homog blea h	0 32	0 21

If the light dapt tion were local the inhomo-g neou bl ch would desensiti only the di tal di eff t ly and thus th displa ement measured wi h homogeneous timul should b le than after an equ l homogeneou bleach Thi i learly not the ca indicating that he proces causing permanent chang pread in the rod A furthe unexpected b rvation i that bl hing 10% pref rentially in the di tal tips ha mu h larg ensitivity-reducing ff than bl hing 10% homogene-ou ly in the rod (bserve that thi i true l when the imulu homogeneou)

The ransient (incompl t) recovery f n-iti y during 30 min aft the bl a h was nearly equal i magnitud when measured with homog inhomog stimul light Thu the proce au ing int rmediat adapt tion lso preads withi the out gme t

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Ki sling, K. H (Depa tment f ani al nut i t on The Swed h Un reity f Ag icult ral Soiences Sweden) EFFECTS OF ETHANOL CONSUMP TION ON MITOCHONDRIAL STRUCTURE AND FUNCTION

The rat f thanol met bolism i th li might b ffected by the act ty f en yme idi i g thanol the act ity f system transpo ti g educing equ alent i t the mitochond i and th bility f ito ho d to d these reducing equival t

Th t bolism f thanol i t f re ith sev ral itochond ial function g the i d ti f f tty ac d d the ac ty f the t acid ycl The ffect probably caused by the l redo tat produ d by ethanol xidation I ontra t t ac te thanol into ication hron thanol onsumpt on soc ted th ultra tructu l chang in th tochond i and inc d mit hond al fra gility F th rmore hro thanol ing tion also ause lt ration f tochond l truc t re nd funct on i ti su wh re thanol not d ed g brain sk l t l mu l and pancre Thu the hron onsumpt f thanol appe rs t produc p tent hang tochond independent f th ac ffect p oduc d y the t bolism f thanol

Th l tochond i th jo p t f the ac tal ehyde f rmed f om thanol The ac t ldehyde lev l the blood increases and ac tal ehyde ha been shown int f re th mitochond al f nct on ou ti su

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Hillbom, M (Research Laboratori f the Sta Alcohol Monopoly (Alko) and Department f Neurology University of Helsinki Finland) TOXICOLOGICAL AND NEUROLOGICAL PROBLEMS OF ACUTE ETHANOL INTOXICATION

The lethal dose f ethanol is not fixed value e.g occasionally there are eports f human f talities produced by rel tively low thanol level and conversely f individual surviving exceedingly high thanol concentrations Autopsy findings uggest that thyroid tate may influence the acute toxi ity f thanol bu therwise there i littl evidence to the factors responsibl f varying the lethal dose i humans

The mortali y f ta t thanol poisoning i modified by ge thyroid tat and previous thanol exposure Young ts tol higher dose f ethanol than ld ones perhaps because thei higher total body wate content cause these doses to result in lowe concentr tions f thanol i the body wat I hyperthyroidism the t f thanol equilib tion i ncreased whil i hypothyroidism i is decreased Thi may t l as partly explain why the l thal dose f thanol i differen f hypo- hype and euthyroid ts

If ts re kep in oxi ted fo 3 weeks the maximum tol rable thanol level in CSF is ansien ly inc eased One may pecul te th this is due adaptive fun onal and or ructu l hanges i he nervou issue

Diff ren l thal CSF concentr tions f thanol in hypo- hype and eu hyroid ts sugge t tha the sensi i ity f br ue to thanol may lso be influenced by the thy id ta Howeve ce ebr l irculation nd xygen consump ion f nerve cell mo lso be ak into accoun

Recent inve tigations indicate that thanol derived acetaldehyde is usually not transported to brai i was f rmed there Accordingly acetaldehyde may contribu only lightly if t ll to the cerebral ffects f acute ethanol intoxication

Some epidemiological data sugge t tha occasional thanol intoxication seems t carry an increased i k f ischemic brai infarction Although this does not imply causal lationship it is tempting t specul t that ffects f thanol on hemocoagulation and ce ebral i culation are involved The thanol induced high osmolality f blood may lead t an increase in hema ocrit Thi ffect on the ther hand is known to low down cereb l blood flow In addition i t ascul aggreg tion f i culating ed cells has been observed in the conjuncti f both expe imental animal and man du ing acute thanol intoxication

One may ask does thanol induced ludging f blood hast be f tal outcome in thanol poisoning? Doe plugging f capill ies by aggreg ted red cell induce cerebr l anoxi during i toxi tion? If so doe this ffect contribu the occurrence f cereb l trophy and dementi in alcoholics? Does hanol influence the cell memb anes f cereb l vessel wall nd thereby provok b ai inf rction?

I orde t resolve some f the above mentioned problems both acute nd long- rm ffects f hanol must be tudied One di ection f uch rese rch should be the examination f the ethanol induced fl idization f membrane which i weak i animal chroni lly fed ith thanol the ffects f thi fl idi tion on the function f cell nd its cont ibu ion toxi i y

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Winkler, K (Depa tment of Clini al Physiology Hvidovre Hospital University of Copenhagen Denmark) HEPATIC AND EXTRAHEPATIC ELIMINATION OF ETHANOL IN MAN WITH SPECIAL REFERENCE TO LI VER DISEASE

This communication surveys studies from the depa tment (1 2) f hepati and extrahepati thanol limination t (EER) in liver disease ith comparisons to normal man Results of tandard ingl injection and steady tate in- f ion technique f lculation of EER are compared Hepati EER i det rmined by hepati venous cathet isation

The tandard ingl inj ction technique were timates total body EER by 15 pe cent (p < 0 02) Singl cases f EER determined t high blood concentr tions (about 30-50mmol l 1 1) in man and isolated pe fused pig liver failed to demonstr te an increased EER and the rea son f r the overestimation i thought to be due to uneven di tribution of thanol Thi i substanti ted by the finding (1) that whil the blood elimination curve from normals were rectilinear con iderabl numbe from the patients were upwards concave this being mor pronounced when majo fluid etention was present

Contrary to several earlier reports we find (1) that EER (ingl injection technique) on the verage is diminished in liver cirrhosis (normal 2 52 mmol min 1 SD 0 62 19 irrhosis 1 77 mmol min SD 0 49 22 p 0 001) and that the decrease seems to be correl ted to the degree f live di ease Howeve very considerabl overlap between EER f normal and patients exists

The hepati venous catheteri tion tudies (2) how that extrahepati elimination (cont) (cont) i saturated t the same concentration level that f the hepati and that it constitues 40 pe cent of total body EER. Apparent Km va f hepati as well extrahepati elimination by rough estimate found to be about 0 2 mmol l 1

The ignificance f these basi data will be discussed with ref rence to ethanol kinetic to the se of EER an indicator of quantitative live function (comparison with gala tose limination) nd to the possible ro l f the consid abl ext ahepati elimination in man

Ref enc (1) Schli hting P Møilund-Carl sen P F Chri tiansen E & Winkl K Ethanol and g lactose limination rat as measure f live function in man To be published
(2) Utne H E & Winkl K Hepati and extrahepati limination of thanol i irrhosis To be published

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Gadeholt G, and Mø land J (Department f Pharmacology Institute of Med i Biology Univer ity of Tromsø Norway) EFFECTS OF LONG-TERM ETHANOL TREATMENT ON DRUG METABOLIZING ENZYMES

The hepati microsomal drug metaboli ing enzyme (DME) ar se itive to environment and di t Sever l diff ent model have been used to study ffects of hroni h gh-dose ethanol (CHDE) on DME A nigh di tary i take f thanol may lead to di tary deficiencies nd DME may become depre sed because they depressed by defi ient diet If CHDE never theles is accompanied by increased nzyme acti ities compared to zero time alne untreated ad libitum fed control thanol in duction f DME can hardly be questioned No tarvation effects hould then be pr sent be ca se both g oups have unr stricted ce to food If on the other hand the control group i pair fed diet more palatabl tha the ethanol cont ining diet the control animal will starve in the interval between fi i hing the daily tion nd the ext feeding Star vation ffect may then become pparent in the control group An unbalanced contr l diet could lso contribut to hanges in the control group

If the CHDE group and the pair-fed control group ar compared the differences between the groups may be the esult f ither thanol ffect starv tion or ther control di t ffect If CHDE nd con l i t ha opposit ffects on DME the esul of comparison will be larg difference between the group It is not however permi ibl to ascribe the entire difference between the groups to the inducing ffect f ethanol Reports of ethanol as strong induce of microsomal drug metabolism have not taken into account the pos ibility f control diet effects

Of central interest in drug metabolism are the microsomal systems consisting f cytochrome P-450 and the associated NADPH dependent reductase system Pr perly designed studies indicate that thanol induce speci f cytochrome P-450 with unique properties shown by cyanide titr tion (Comai Gaylor 1973) inhibitor tudies (Ulrich t l 1975) activity studies (Villeneuve t al 1976) nd g l lectrophoresi (Ohnishi & Liebe 1977) The P-450 isol ted after CHDE ha higher turnover numbers for ethanol (Ohnishi and Lieber) nd niline (Villeneuve et al) than P-450 from phenobarbi tal or 3-methylcholantrene treated ts Induction of niline hydroxyl se acti ity can be shown in crude microsomal preps tions together ith increased P-450 One can expect that CHDE rats will have accel ted limination f drug whose maj metabolites ar formed by microsomal niline hydroxylase acti ity

NADPH f rricytochrome eductase i the most importa t electron donor t microsomal cytochrome P-450 Pe unit body weight thi acti vity does not increase compared t ro time lue (in ts) fter chroni ethanol treat ment The increased in ivo limination of drug fte hronic thanol t eatment then probably t least i part sult from the quantit tive and qualitative hanges in the hepatic micr somal cytochrome P-450

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Has inen I Savol inen M , Lahtone M and Hiltunen, K (Depa tment i Medical Biochemi try University f Oul Finland): EFFECTS OF ACUTE AND CHRONIC ETHANOL ADMINISTRATION ON HEPATIC LIPOGENESIS AND TRIACYLGLYCEROL METABOLISM MECHANISMS AND MEDIATORS OF ACTION

Th mechanisms f the development f th cut and chroni th l indu ed f tty li i not known i det il t th nzymi l vel Although extrahepati f ct may ont ibut t the development f thes condition most f th data g thered hith rt suggest f p ima ily hepati derangems ts

Th f tty li d velopi g ft ingl l rg dos f than l i t du t enhanc d hep ti f tty id ynth judged from dat from xpe ime t in vivo and i pe fused li Th i no viden f d cre d rat f lipoprot in tion ith Th gly rol 3 ph sphat cylt ansf how q t limit d capa ity f long t rm gul ti and glycerol 3 ph sphat ailability ha been ggested as mechanism f regul tion f f tty id t ri fi tion

Phosphatidat ph phohyd l has th lowe t cti ity f the en yme f the t i cylgly l synth i i g p thway and its ti ity changes i pons t di t ry met bol man pul ti Moreove th cti ity f ph sph tid phosphohyd las increases t three-f ld value ft an cut than l loadi g (1) The mech ism f thi i creas remains to be t bli hed The ffect i not direc ly linked t than l met b li es becaus th ctivity cann t be in cre ed in an i l ted perfus d li Howeve unde lim t d ondit ons the phosph tid t phophohydrolase ctivity hows posit ve rel tion with th h p ti glyc rol 3 phosph t con ent tion gg ting that small-mol cul weight met bolit may be invol d in the gu l t on f thi zyme (2) Cortis l induc ph sph tid t phosphohydrol se by ds ti ony i nsit ve mechanism i i anl in i l ted perfus d li rs (3) and denc ha b en p ented f increas d plasma cort l l vel aft cut thanol l adi g Th dat gg t that phosphatidat phosphohydrolas may be ne f th k y en ymes i the thanol induced cumul tion f f t in th live

Th mechanism f the developme t f the h on i than li f tty li y b p rti lly dif f t Und controll d ondition specifi ff ct f than l on f tty id synthesi dur ing long t rm then l dmi i t tion can be rul out (4) Mo eov one f the cetyl CoA and NADPH-gen rati g en yme how d reas d ti it ft ontinued thanol dmi ist tion The i reas in phosph tid t phosphohydrol se ft an cut thanol load lev l p rti lly ff duri g continued th nol dmin- l tration The view emerges that during ch on than l int k the rbohydr t nd lipid on tent f th di t ffects the hep ti lipogene- i nd lipid ccumul t on E t mation f the ont bution f the variou enzyme ti ity hanges during long t rm xpe ime i mad diff cul by the rap d tt lmme t f t dy t t lipid on t tion i the li er

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Öbrink, K.J. (Department of Physiology and Medical Biophysics University of Uppsala Sweden) THE ANIMAL AS A SCIENTIFIC INSTRUMENT

All esearch is depending on different kind of instruments In biology and medicine the laboratory animal is very important instrument both in quali ative and in quantitative analysi As for any other type of instru ments this one mus be encitive have high preci ion and be reproducable For normal physical instruments like balance photometers etc thi ha always been selfevid nt but for the laboratory animal but has unfor tunately not been The sci nti ha been forced to be content with widely scattered results Thi has mostly been blamed biological variability but i many case the blame i to be put on the scientist himself

W must remember that living being has homeo- tatic mechanism by which i trie to keep its internal milieu constant W have control mechanisms for body temperature blood pressure blood sugar number of blood cells and practically all other parameters in the body As soon as something intends to interfere with thi quality the body has the ability to counteract in order to restore the original situation. Thi property the mos remarkabl difference from physical instruments.

W may call all the factors that i some way inter fere with the constancy of the body parameters as stre factors Thi is wide definition and will in clude actions of microorganisms like bacterias and viruse and from parasites I also include the ffect of the nutrition which constitutes heavy load on the body It include all types of invironmental factor not only physical ones like emperatur humidity light and so forth bu also social factor like number of animal in the cage relations betwee the animals and the caretakers tc All these parameters will be important for the xperimental esult and may introduce erious error A general rule must be to keep the parameters constant and if pos ibl under control

Let us take an example In homeothermic animals the body temperature is kept constant even if the ambient emperature change It is quite obvious that two genetically identical animal may keep the same body temperature and till be quite different because one animal may have to defend its temperature against cold while the other is sitting in comfortable environment They use different amounts of their regulatory capacity and that makes them respond differently to the xperimental ituation The results will be accordingly

It is imperative that the scientists are aware of the above facts and it is equally important that all categories of people handling laboratory animal must by well trained Finally the quality of the animal their micro- biological statu the animal quarters and the husbandry mu t be of acceptable quality

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Carstensen, J (Inst of Toxicology National Food Inst DK 2860 Søborg Copenhagen Denmark) THE NEED FOR BETTER SPECIFICATION OF THE ANIMAL MODEL IN THE TEST SITUATION

It i essenti l to realise and carefully evaluate ll the factors which may ffect animal studies By ignoring the potential impact of an erroneous extrapolation incorrect conclus ons may be the result Thi will make the scientist vulnerable to scientific criticism and from an ethical point of iew the scient st may be blamed for u-ing animal for worthless experimentation

In general the specified animal model offers the fol lowing advantages Reliab lity reproducibility lower number f animals reduced time and cost Fewer errors in extrapol ting to the same or other species including man

An important but lso depressing survey has been published by Lang & Vesell (Fed proc 1976 35 1123) They summe ize the description of the animal models in 4080 articles from 7 selected bi logical journal i percent species 99 8 train 58 3 source 24 1 sex 45 5 age 19 4 bodyweight 50 3 popul tion density 8 0 cage type and size 7 6 cage lean ng 0 3 bedding mate i 1 0 9 type of food 26.6 feeding frequency 12.6 temperature 6 5, humidity 1 2 ventilation 0 2 photoperiod 6 9 conditioning 9 2 selection 4 9 handling 0.6

Thi illustrate the poor background information for animal model normally given in the literature Also the test situation in itself call for clarification i the publications Examples ill illustrate this

Da ly and seasonal vari tions Body temperature blood pressure and blood constituents va ies during the day Hormone balance and behaviour in females varies du-ing the estrus cycl Cortisone induced l ft pal te i mice show variation during the yea T ll when the study was made

Blood sampling Blood cells enzymes and hormones may vary cons derably between sampling ites and ith sampling technique T ll where when and how

Urine sampling Sampling time relation t the possible dosage regimen time interval ince the previous vei ding f urine or sampling should be indicated

Anaesthesia An anaesthetized animal i very special model Measurement and analytical data are in many in-tances representative for the anaesthetized animal only Specify les ly

The test substances Should be well defined have specificat on stating the degree of purity and remaining material must be charact ized q intermediates and impurities from the ynthesis

Pharmaceutical and other preparations How i the preparation made g ve name og regi tered formul tions and identify pharmacopoei preparations Bioavailability is very important constituents may bind the active compound

Administration of compounds and preparations How and where t administ red Give concentration and volume of solutions as it has an influence on the resorption from the application or injection ite Oral intubation or d etary inclusion do not give identical results

Postoperative care Scient st should pay much more ttention to this Proper surveillance and instructions re important to ensure good result After anaesthesia when curare like effect may still persist the injection of antibiotics may act ynergistically and even kill the animal Describe treatment

Euthanasia T ll procedure and time f killing

Organ sampling Describe handling of animal immediatly before killing the sampling technique and time sequence

Apparatus/equipment Substances likely to migrate from plast tubes bags and catheters may be toxic and interf re with measurements Give brand name/quality

Conclusion. These and other parameters re included the recommendat on from the International Committee on Laboratory Animal (ICLA Bull 1978 no 42) Thi recommendation is an important check list for ll authors as well as f the editors and reviewers from bi logical journals

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..artne , K (Laboratory Animal Centre Medi l School Hannov (Germany) HANDLING AND EXPERIMENTAL PROCEDURES FREQUENTLY OVERLOOKED AS STRESSORS AND SOURCES OF TECHNICAL ERRORS IN EXPERIMENTS WITH ANIMALS

Blood hemi l and physi logi l cha ac t ti in laborat ry animal show gre t rianc i group f tand rdi ed animal Thi i l rg ly due t the indi idual component f ianc and may be aused by
1 The resso pacity f normal handli g and xperime t l procedure Thi was in tig ted by rec rding th time rse f 25 endoc ine irc l t ry and me boli ha ra t i ti i rat ft diff rent tress-so confront tions (g ran port tion handling the tress)
2 The limati on proc ss fte pu hasi g and transpo ting labo t ry ani mal Thi was i ve ig ted i ch act i i f qui k (re) d y and del yed (immunologi al food) up t 100 day acclimati tion
3 The type f blood sampling procedur timat d by omparing he procedur fluenc on blood nzyme on blood hormone l l and on the oncen i n f small mol cul i blood i re

The i tenc f l d an rhythm (4-6 hours) f met boli linked ch act i ti tabli hed by t leme i lly rec rd g the be t rat the body tempera re and he moto -ac i ity i undi turbed observ d ov long pe iod f time

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Obrink, K.J. (Department of Phy iology and Medical Biophysic University of Uppsala Sweden) SOME ETHICAL ASPECTS ON ANIMAL EXPERIMENTATION

The important que tion whether or not the man ha the right to us animal for his own purposes will not at all be discussed here W may consider that question answered by the mere fact that we are using animals in biological and medical re earch It i however impor tant to remember that thical problems ar by no means solved by thi tatem nt Instead ethical problem is judgement between the everity of the experiment on the animal and the purpose of the experiment It mu t be considered unethical to make an experiment on an animal if there i no sound scientific problem behind the experi ment Decision in ethical problems is judgement of the relation between severity and purpose Up til now the scientist himself has made that decision Probably most scientists have fulfilled that responsibility with high thical tandard It is however always risk that pecialist or any prof ssional gets blind for what he i doing i hi daily work and therefore it would be an improvement if he would discus hi experiments with some f llow-creatur before the tart of the xperiment

I Sweden ethical committees for hat purpose have bee establi hed They try to be of help to the scientists without bureaucracy In each judgement the members of the committee must convince themselves

that the project i sci ntifically sound and that it is not an unnece sary repetition of already made experi ment

that the problem cannot be solved without the use of animal nd

that the periments cannot be modified so to involve lower degree of animal di tre

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Fi ne H od Sand T (I tit t f M dical Bi logy U iversity f T omss Norway)
CHARACTERIZATION OF THE HEART VENTRICLE BY ITS PVt PLANE

We have searched f load in riant desc iption f the pe formance f th heart Th ight ent i l f is lated oat he t pumped t const t end-diast li (ED) pressure (P) gainst va iabl hydro lic art ial load Th re istanc nd complianc f the load co ld be ried on st dy tat ba is; we al had means f ingl beat iation V t i ular P ad flow (F) were recorded nd ve t i lar olume (V) wa computed by flow integration assuming constant EDV

Three-dimensional diagrams f P V and time after onset f cont action were constructed The three va i bl f llowed trajectory in pace which d pended on th t ial load Howev b th i th ce f teady tat load ari tions and during ingl beat load chang the traj ctories l sely f llowed singl surface which we denot the PVt plane Th surface i ind pend t f th load it i the f re measure f th int in i heart pe f rmance Diff rent heart had diff rent PVt planes and inotropic int rv tion had marked nd haract isti influenc on th shape and position f the plane

Fo y gi load the PVt pl ne can be med t comp te as f tion of time entri ular P F V any l ted qua tity W ha q it occur t ly econst ucted P and F urve i our pe ime t

Th PVt plane is compl t paramet free repres t tion f th tat f the heart ntri l W sugg t th t th PVt plane is pre ent tion perceding the mo t f diff rent way f pre ting t i l pe f rmance

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Piene H od Sund T (I titut f Medi l Bi logy U iversity f Tromsø, Norw y)
MATCHING BETWEEN RIGHT HEART VENTRICLE AND ITS ARTERIAL LOAD

Th ight vent i l (RV) is traditi nally consid red t be flow pump This should mean that trok powe in reases directly i proportion t th load re istance Thi charact i ti f th RV was tested f in isol ted cat hea t pumping ag inst load f va iabl re ist nc (R) nd compliance (C)

RV mea flow decreased ith incres i g R d cre ing C F const t C ptimum in trok powe was f und t certai alue f R Ropt F high C (5 10-5 dyn-1cm5) R pt wa ~10 10+3 dy cm 5 whi h is i th high normal rang f pulmonary ascul re istance When C was lowered R pt shifted t lowe lue Pl ts f mean RV pres ur flow yi lded traight line whos l pe ca be tak th apparent source re ist nce Rs f th t i l Rs wa found t be bout 3 5 10+3 dy cm-5

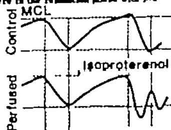
From theoreti l con id ration we bt ined th f llowing relation b tween Ropt and R f C→∞ R pt Rs yol ti / yst li time; for C→0 Ropt Rs

W conclud tha RV i not flow pump b t mat hed t th re i t nc f the load Th load complia ce i it wiadk ssel property fl enc the matching i su h way that it f voured by high compli nce t normal l f pulmonary ascul re ist nce

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Ilebekk A., Lekven J, and Kiil F (Institute for Experimental Medical Research University of Oslo, Norway).
ASYNERGIC MUSCLE FIBRE FUNCTION IN THE LEFT VENTRICLE DURING LOCALIZED INOTROPIC STIMULATION WITH ISOPROTERENOL.

The purpose of the present study was to examine muscle function in the left ventricle of anaesthetized open-chest dogs when localized part of the muscle mass was selectively stimulated with the β-adrenergic agonist isoproterenol. A shunt line was established between the left carotid artery and the anterior descending coronary artery for infusion of isoproterenol. One pai of piezo-electric elements was inserted into the rea to be stimulated ith isoproterenol and anothe pai into contro rea. T examine the contraction pattern the distance between the two piezo-electric lements the chord length (MCL) was continuously recorded by an ultrasonic technique to estimate muscle fibre function. Small doses of isoproterenol (0.1–0.8 µg, min) hich induced no general haemodynamic effects changed the contraction pattern of muscle fibres both in the stimulated and in the control area. In the control rea, muscle fibres were stretched in early systole due to the greate rate and magnitude of tension development in the stimulated than in the non-stimulated muscle fibres. When the control rea was in late systole isoproterenol-stimulated, muscle fibres were stretched because they already were in the relaxation phase with low tension. On the other hand the control area contracted to lower MCL. Hence administration of isoproterenol to part of the left ventricle results in asynergic contractions and reduces the overall performance so that stroke volume may not rise despite extensive inotropic stimulation.



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Mills M M., Geiran, O., Ilebekk, A., Stokland O Thorvaldson J and Kiil F, (Institute fo Experimental Medical Research University of Oslo Norway)
FUNCTION OF THE INTERVENTRICULAR SEPTUM DURING VOLUME LOADING OF THE RIGHT VENTRICLE

The influence of the interventricular septum on left ventricular function was studied in 7 anaesthetized open-chest dogs during volume loading of the right ventricle. Muscle fibre function in the septum and in the free walls of the right and left ventricles was estimated by implanted ultrasonic elements The distance between the lements is called myocardial chord length (MCL). Volume loading of the right ventricle was achieved by opening shunt between the pulmonary artery and the vena cava. By opening the shunt right ventricula stroke volume rose by more than 60%. End-diastolic MCL and systolic myocardial shortening (MS) in the free wall of the right ventricle and in the septum rose whereas end-diastolic MCL and MS in the left ventricular free wall decreased. Left ventricular stroke volume fell by 7 ± 1%.

T determine the significance of stretching of the muscle fibres in the septum during right ventricula loading for left ventricular performance, vena cava was constricted until end-diastolic MCL of the left ventricula free wall was reduced as much as during volume loading. By vena cava constriction end-diastolic MCL of septum and free wall fell in proportion, and left ventricula stroke volume was reduced by 13 ± 1% which is significantly more than 7 ± 1% (P<0. 001).

Thus volume loading of the right ventricle activates the Frank-Starling mechanism of the septum. Thereby the performance of the left ventricle is also improved.

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Stokland O, Miller M M, Ilebekk, A and Kiil F (Institute for Experimental Medical Research University of Oslo Norway) MECHANISM OF HEMODYNAMIC RESPONSES TO OCCLUSION OF THE DESCENDING THORACIC AORTA.

During acute occlusion of the descending thoracic aorta systolic elevation of left ventricular pressure (LVP) is associated with dilation of the left ventricle. This dilation is usually attributed to retention because of high afterload. However the rise in systolic LVP may on the contrary be dependent on the increase in left ventricular preload. T examine this hypothesis myocardial chord length (MCL) was measured in the anterior wall of the left ventricle by an ultrasonic technique in nine atropinized open-chest dogs. During aortic occlusion end-diastolic MCL increased by 14 _ 8% ystolic LVP rose by 84 _ 8 mmHg. Stroke volume did not decrease because blood flow in the superior vena cava (SVC) more than doubled. By simultaneous occlusion of the inferior vena cava (IVC) and aorta redistribution was prevented and systolic LVP and SVC-flow remained unchanged while end-diastolic MCL decreased below control. By blood volume infusion into the jugular vein during combined aortic and IVC occlusions end-diastolic MCL, systolic LVP and SVC-flow increased as during aortic occlusion alone. Control values were re-established by withdrawal of 7-8% of total blood volume. Hence during aortic occlusion the left ventricle is dilated because of redistribution of blood volume from the lowe part of the body the Frank-Starling mechanism is activated stroke olume maintained and systolic LVP increased.

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Ihhd , ? and R j selmi M, (Departments of Radiology nd Cardi logy Uni ersity og Oul Finl nd): NORMALIZED LEFT VENTRICULAR STROKE WORK AS AN INDEX OF MYOCARDIAL FUNCTION

Th purpos of this t dy wa to evaluat th e nsiti ity f diff re t ejection pha i di es to v rify functional abnormalities of th l ft t i l Th mat ri l consisted f qua tit ti l ft vent icul cineangi grams nd si ult eous pressure recording f 24 pati ts with orti valv dis bef re and 12 nth ft val pro thesi ins rtion nd of 22 patient without org ic heart dise A gre t umbe f param ters we checked a- ng tho l ft vent icul troke work di vided by both l f entricul end-di tolic v lum nd mas (SW/EDV/mass) in rde to e- liminat th improving ffect of both high stret hing tat nd big mas on th myocardi l p rf rmanc I this pape we present th result onc rning SW/EDV/ma s jecti n frac- tion (EF) nd ircumf renti l fiber shortening locity (V f)

SW/EDV/mas pro ed preoperati ly lowe tha th normal val (41 µJ/ l/g 19 52 µJ/ ml/g 19 respecti ly, p<0 05), improved post- p ti ly (66 µJ/ml/g 26 p<0 001) nd did no r ignifi tly diff from normal EF (p ent) a p eope ti ly 60+1 postoper ti ly 66+10 d in co trol group 65+7 The rre ponding al es f Vcf (circ/s) were 1 9 1 0 2 0+1 0 nd 1 7 0 5 re pectively N 1 The th ch ng th devi tion from normal f EF nd Vcf ea hed th t tistical ignifi canc Th SW/EDV/ mas f th first time used in thi st dy proved sensiti rd the post- op rati improveme t wa in good rrel tion with functional improvement f th patients

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Pl E S Refsum H and Mid O D (Insti of Medic l Biology Unive i y of Troms) ELECTROPHYSIOLOGICAL EFFECTS OF ISO- ...OL NICOTINIC ACID AND FREE FATTY ACIDS IN THE DOG HEART IN SITU

C t hel t known t h hyth g i ti Th p t t dy don to t t if th ti ight b di t q enc f s d ta h l i ti ty di ect ff t thro gh i d pl s f f tty id (FFA) d t d by th t h l i Sev i t t p t b rbit l th ti ed d g d Pl ce t ti f FFA we i d by i dn i tratio f is p t nol (0 15 g/ i kg) Th i i pl a FFA ld b b li hed by i i f f i ti i id (0 3 mg/ kg) Mi h di re d nge d d f t i e d by f prog mm d lect i l timul ti Th q nc f eqi t ti 1) t l I 2) prot nol I f lo 3] t ol II 4) ti i id i f f 5) i t id pl prot I I prot l d d pro ed i c s i th h t t d dec i th inus od ec ry ti Ni ti id gi ith i p t l d ed lightly thes ch g hil i ti i id l h d ig iff t ff t I p t nol d nd t i l AY d l d t l f t i s ll s th AY d ti t whil i ti i a id had infl nc th i bl al se i corbi ti ith i p t l

The p t t dy h lthy dog i d t th t i p t l ha d t rhythmog i ffect hi h ay be i d p nd t of h g s i pl a FFA Th t dy f th i di tes th t icati i id ale h mo di l tro physi l ff cts

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Kuikk , J T Paikka E O, and Bas ingthwaight J B (Departmen of Clinical Phy i logy Uni ver ity Central Hospital Kuopi Finland and Center fo Bioengineering University of Wash- ington Seattl U S A) HEART CAPILLARY PER- MEABILITY TO GLUCOSE MOLECULES

Bolus injections f ref rence tracer (131 I RISA) and test ubstance (2-deoxy-D-3 H-glucose D-3-H-glucose or D-14-C-glucose) wer made i to the coronary art ry inflow f isolated blood-perfused dog hearts 23 pairs f trace dil tion curves were ecorded from the coronary inus outflow with plasma flows F ranging from 0 33 to 2 50 ml/min/g

Capillary permeability-surf ce ea prod- ucts we estimated from insta taneous extrac- tions PS_E and from capillary interstitial- cell model PS_M Peak extractions ranged from 0 14 to 0 50 and PS_E was on the ve age 0 61 0 22 ml/min/g (mean S D) and PS_M 0 73 ± 0 27 ml/min/g An exponential relationship was found between the PS-determinations PS_M/F 0 10 exp(3 7 PS_E/F) 0 97 N 23

Effects of flow F haematocrit HCR and lative spati l di persion of lbumin transit times RSD on PS_E and on PS_M we made using multilinea regres ion analysi Correlation coeffi ients between variables we

	F	HCR	RSD	PS_E	PS_M
F	1 00	−0 61	−0 42	0 44	0 01
HCR	−0 61	1 00	0 51	−0 02	0 33
RSD	−0 42	0 51	1 00	−0 23	−0 03
PS_E	0 44	−0 02	−0 23	1 00	0 80
PS_M	−0 01	0 33	−0 03	0 80	1 00

We concluded that F HCR nd RSD have no ignificant ffects on PS_M whe eas PS_E depends on F and PS_E underestima es highly PS-products at high extraction nd t low flow levels

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Sel th O R f M , Vik Mo H d Mj s Ø D (I stit te of Med l Biology U i sity of Tromsø Norway) MODE OF ACTION OF HYALURONI DASE IN ACUTE MYOCARDIAL ISCHEMIA IN DOGS

I d te t dy th echa is by whi h hyal idas red s my ca di l i hemi inj y thre p t d c o y t ry l i ve p f rmed i 12 p h t stb tiz d dogs T l ic th high pl s con t ti f fre fatty id (FFA) s lly b rv d i t myocardi l i f ti l ma d en 11 (0 125 µg/ i kg) i f d i d i g th 2 d a d 3rd l i Hy luro id 225 NF units/kg wa gi i 20 a b fo the 3 d eccl l I hemic i ju y s d a th em f ST egme t l tio (IST) i pi di l ECG re di g from 10 16 it ft $ d 10 i ec l si s R gio l myocardi l bl d flo d t rmi d si g r di l belled ic ph r Bl d sa pled from o t ( ) ry si (cs) d from lo l si (l) drai i g i hemi ti d s d a l diff f gl l ct t FFA d O_2 were d t rmi ed Hy l ro idas treatment red d IST from 44 7 ± 0 to 34 9 ± 6 5 V (me SEM, p 0 005) t $ l ccl sion a d fro 48 4 ± 6 5 to 39 6 7 l V (p 0 005) t 10 i o l lo Th s 20% l s (p 0 01) l i hemi epi rdial blood flow ft hy l ro id tre t ment but o ig ifi t h g s i flow i th ischemi d c rdium Hy l ronid h d ig ifi t i flu y rdi l flu f b t t s d O_2 o tb i hemi a I cl f hy l ro id d ed i h mi i j ry d i g dren l i f i Thi d tion may b pl f d by i c ed ll t l bl od flow d t by lt red h g f h t t cro th i hemi my dium

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Hedberg A-1) Minneman K P, and Molinoff P, B 2) (Dept f pharmacology AB HH l Molndal Sweden 1) University of Colorado Medical Cente Denver Col rado, 2) DIFFERENTIAL DISTRIBUTION OF β_1 AND β_2-ADRENOCEPTORS IN CAT AND GUINEA PIG HEART

Accumulating evidence indicates contradictory to the concept f Lands (1967) coexistence f β_1 and β_2-adrenoceptors in the same organ (Carlsson t l 1972) A difference in the β_1/β_2-rati between the SA-node and the ventri le in the cat heart has also been discussed (Carlsson et l 1977) In the present tudy ix different β_1 or β_2-selective ligands were used to inhibit the specifi nonselective binding f ^{125}I iodohydroxybenzylpindol l in crude membrane preparation f right trium (RA) and l ft ventriol (LV) f the cat and guinea pig heart Analysis f the data according to Hofstee revealed biphasi displacement kinetics indicative f binding to two receptor sites presumably β_1 and β_2 The regional mean relative densities f β_1 and β_2-adrenoceptors calculated from values obtained with each competing ligand were as follows

	CAT		GUINEA PIG	
	RA	LV	RA	LV
% β_1	78 1	98 0	76 7	100 0
% β_2	21 9	2 0	23,3	0

The difference between RA and LV densitie f β_2 adrenoceptors proved to be significant when calculated femtomoles receptors/mg prot The concentr tion of β_1-adrenoceptors in the cat LV proved to be nearly twice that in the guinea pig LV whil there was very little difference in trial β-adrenocepto concentr tion between the two species

Our data thus support the findings from the pharmacological in vivo experiments in cat

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Skomedal T , Osnes J -B and Øye,I (Instit te f Pharmacology Uni f Osl Norway) ALPHA ADRENERGIC BLOCKADE IN RAT HEART BY PRAZOSIN EVIDENCE FOR TWO ALPHA ADRENERGIC RECEPTORS

Phenylephrine (PE) in presence f prop anolol (PRO) evokes an -adrenergi inotropi response in mammalian heart The time-cou se f this spons i characte ized by transien decrease i maximal developed tension (T_{max}) f llowed by lowly developing increase which reaches maximum after 4 5 min In t papillary muscles PE ($5x10^{-5}$mol/l) in pr sence of PRO caused transient decrease i T_{max} to 88 6% f control f llowed by an increase t 136 9% of control

Prazosi (PRZ) which i believed to be selective blocker f postsynaptic α-receptors displaced the dose- esponse curve for T_{max} and dT/dt_{max} to the right (pD_2 value 0 53 and 0 57 espectively p<0 01) without change in maximal ffect PRZ thus behaved as competitive antagonist with high affinity (inhibition constant ~ 4 10 $^{-11}$mol/l pA_2 ~ 10 4)

The ffects of phent lamine (PT $6x10^{-7}$mol/l) and PRZ ($6x10^{-9}$mol/l) on the time course f the -response differed qualitatively PRZ blocked the positive component only (111 5% of control p<0 01 compared to PE) f the inotropi re ponse whereas PT inhibited both the nega ive (93 1% f control p 0 02 compared to PE and PE PRZ) and the positive compone (110 4% f control p<0 01 compa ed to PE)

These data may indicat tha there re both inhibitory and excitatory α-adrenergi receptors in t heart located postsynaptically The excitatory receptors may be 1 due to the preference f PRZ whil the inhibitory ones may be 2 due to the non-selectivity action f PT

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Kettunen R Timisjärvi J , Kuikka J and Hirvonen, L. (Department f Physiology Un ve sity f Oul Finland) EFFECT OF EXERCISE AND SMOKING ON BLOOD CIRCULATION ASSESSED BY RADIOCARDIOGRAPHY

Radiocardiography provides saf non in ive tool for determining functional blood vol umes flows f systemi and pulmonary ircuit cardiac chamber volumes and for the mathemati al estimation f pulmonary arteri l mean and capillary pressures permitting also exposure to various stimuli such xercise and smoking We carried out an indium or technetium adiocardiography on 60 volunteers (age 20 to 62 yr) t rest and during upine bicycl ergomete exer ise with and without smoking At rest the results btained by repeated ecordings were equal Smoking raised heart rate and ardiac output but did not cause furthe ignificant changes Two diff rent exercise serie were carried out 1) three repetitive recordings with the first at rest and the following t two level f exer ise 2) two repeti ive recordings with an in terval f 30 min the second wo k load preceded by smoking In series 1 cardiac output in creased up to 220 % strok volume 30 % ject ion fraction 15 % and pulmonary blood volume 30 % The change could be depicted by polynomi l function with heart at as the absci Pulmonary capillary pressure decreased when cardiac output increased In se ies 2 heart rate and pulmonary capillary pressur ttained ignificantly highe values fte compared to before smoking whil decrease was observed in stroke volume pulmonary blood and dispersion volumes The conclusion i that smoking impairs phy i al performance increase pulmonary capil lary pressure decrease pulmonary blood volume and probably the number f open capillaries

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Torma M., Sakki, S., Vähätalo S. and Härkönen, A. (Päijät-Häme Central Hospital, 15850 Lahti 85, Finland): LARGE FIELD GAMMA IMAGING IN STUDIES OF CERVICAL AND CEREBRAL CIRCULATION:

With a standard field gamma camera it is not easy to study the anatomic and functional tatus of the cervical and cerebral circulation simultaneosly

In order to examine the arterial circulation from aorta via cervical arteries to hemispheres in the same study a large field (∅ 40 cm) gamma camera was used A bolus injection of 15 mCi 99m-Tc was used By aid of a computer it was possibl to get a visual impre sion of the status of various vessels in different phases of circulation The regional circulatory curv were written bilaterally from the areas f common carotid arteries and erebral hemispheres The circulatory time from the aorta to the convexity was calculated too This offered valuabl further information especially in the diagnosi of cerebral AV-malformations and infarctions The correlation of large fi ld gamma images with x ray angiography was good

Conclusion: The dynamic imaging of blood flow from aorta to cerebral hemispheres i possibl with a large field gamma camera By aid of the computer technique it is possible t get a visual impression of th status of various vessel and to calculate dynami circulatory curve from the regions f interest The large fi ld gamma imaging seems to be a suitable noninvasive method for a routine is tope laboratory

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Timisjärvi J and Hirvonen L (Department of Phy i logy Unive ity f Oul Finland) THE BLOOD CIRCULATION OF THE REINDEER.

Right heart catheterizations with the recordings f i gle-plane cineangiocardiograms and blood pressure were performed on 25 reindeer aged from 5 to 18 months The calculation f l ft ventricular volumes utilized cut-down evolution llipsoid as geometri reference and two epar t regression lines for volume correction The left ventri l contracted like blacksmith bellows Stroke volume was 2 4 ml/kg cardiac output 130 ml/kg/min jection fraction 76 % heart rat 80 b/min aorti blood pressure 153/130/115 mm Hg and pulmonary arte-ial pressur 25/16/11 mm Hg in the prone posi tion t rest With the animal on it ide pul monary art ial pressure increased by about 10 mm Hg, rdiac output and stroke volume by 25% Art rial hypoxia incr sed heart rate aortic and pulmonary rt ri l blood pressure end-dia toli and str ke volume and ejection fraction If the irculatory variabl were taken as discrete groups correlation between them could be calculated g second power polynomial co l tion between) heart rate and aorti blood oxygen tur tion; b) heart rate and left ventricular volumes;) aorti blood pressure and left ventricul volumes; d) aorti blood xygen de turation and pulmonary arteri l pressur tc In these experiments the reindeer cardiorespir tory system eacted readily and apidly to various timuli Its blood oxygen carrying capacity was high (Hb 180 g/l and blood volume 106 ml/kg) and there exi ted high degree f cardia reserves The pulmonary i culation ppeared to be suitable model for studi f pulmonary hyperten ion

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ilainen T O A (Department f Pediatrics rsity Oul Finland) VECTORCARDIOGRAPHIC PATTERNS IN INFANCY NORMAL VALUES AND INDIVIDUAL VARIATION

Frank y and leads were recorded from 62 he lthy full-term infants during the fi st 48 hour and t the ge f one week 1 2 4 6 9 and 12 month with the equipment developed by Arvedson The planar and patial QRS loops were analyzed by computer The mean lue f ach 5 ms instantaneou vector and their 5th 10th 90th and 95th percentil were computed The maximal spatial ectors to the right and to the l ft spatial mean QRS and ST T vectors and spatial angular devi tion f T_{max} from QRS_{ma} wer computed

The mean horizontal loop rotates lockwise At the ge of one month it f rms figure-of ight late i otate coun rclockwise

The initial vect rs re directed ant rio ly t the l ft but fte the ge of one month to the right Their magnitude incre se during the first 6 months The di ection f 35 and 40 ms vectors change very considerably during in fancy from f ight to far l ft Thei magnitude increase with ge There is littl change i t rminal ectors

The individual variation f the ini ial vect r and 35 and 40 ms vecto i very large and quite hig in most ther vecto The values f Azimuth ary more than the lue f levation and magnitude

It seems that cardiac lectromotive f rces change quite individually in time and in space during the first ix months in i fancy ith great va i tion of normal value

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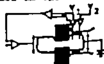
Swenson, M (Department f Biophysics Univer ity f Copenhagen Denmark) MEMBRANE POTENTIAL CONTROL IN MAMMALIAN HEART MUSCLE

Voltage clamp tudies on ardiac muscl ti lising the sucrose gap technique are complicated by diffusion in the extracellular space of sucrose into the compartments perfused ith physi logical alt solution and vice versa

Anothe complication tems from the superfi cially located cells in the potential control led part f the preparation having smaller eries resi tance to ground than the deeply located cells Thus radial potential g adients in the extracellular pace will compromise the homogeneity of the potential control

Experiments on papillary mus le or trabe lae carneae from guinea pig cat pig and dog reveal that the sucrose gap can be replaced by controlled localised compression of the extracellular space effectuated by reducing the diamete of hole in 5 mm thick rubbe partition through which the preparation has been pulled This way the longitudinal resistance can be increased t l st 10 f ld before the intracellular resistance is influenced

Radial potential gradients in the xtracel lular space of the potential controlled end f the prepar tion are diminished by reducing the amount f the surrounding salt sol tion to thin (10 µm) flowing layer creating an artificial extracellular pace on the surface of the preparation With one f the current lectrode positioned t the end f the preparation superfi ial and deep cell will have equal membrane current density (I_m)



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Peuhkurinen, K T kal , T , Hiltunen K and Ha
iinen, I (Dept t Medical Biochem Uni rsity f Oulu, Fi land) EFFECTS OF INTRAVENTRICULAR PRESSURE ON THE TRANSMURAL DISTRIBUTION OF TIS SUE OXYGENATION IN ISOLATED PERFUSED RAT HEART

NADH and fl vin fluorescen can be us d as probe of the ll l oxidation- duction tat The ffect f i traventricul pressure glob l l hemi and oxyg diffusion from int en tricul spac on th transmural distribution f tissue oxygenation we examined i potas ium rrested Langendorff p rf ed t heart by NADH and flavi fluorescen photography t liquid itrogen temperatures Cro ect ons from quick fro en hea ts were bt ined by filing The wave length f th ex it tion light as 360 nm f NAD(P)H and 465 nm f flav Th came was guarded for the ex it tion light by suitabl filters When th intravent icular p es re was ro and th orti pressure 7 85 kP the cros ecti ns f th myoca dium wer unif rmely oxygenated When the i t aventricula p su ppro ched the orti pressure (6 86 and 6 85 kPa respec ti ly), an anoxi on was b erved in the sub endocardi l laye d t ct ble by i tensi NAD(P)H fluores enc and los f flavin fl es ence The immermo t subendocardi l lay emained, however oxygenated p obably by dire t oxygen diffusion f om th tra entricul sp ce I gl bal ch emi aused by lowering the ti pressu t ere only th out rmo t p rt f th subep ca d l layer em ined oxygenat d probably by direct diff ion from t

When anoxi on we b rved they lways demonstrated every harp bord between anox and oxygenated eas Th esult lso ind t than in tudy on rre ted heart , car mu t be t ken t dj t th travent cul pre su t low l t avoid uneven myocardi l pe fus on

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Takal , T and Ha inen, I E (Department f Me dical Biochemistry, Uni rsity of Oul , Finland): EFFECT OF MECHANICAL WORK LOAD, ENDDIASTOLIC VENTRICULAR PRESSURE AND CORONARY RESSURE ON AMINO ACID INCORPORATION IN TO LEFT VENTRICULAR PROTEIN OF ISOLATED PERFUSED RAT HEART

Th mediato f th ffect f p ure lume ov load on myocardi l prot i synth i not known but mechanical wo k myofibrill di tention hemical medi t may be invol ed Langendo ff-p rfu ed t he rt were us d t e- pa t ly tudy th ff ts f mechanical wo k onary pre sure and end-di t li entricul p essur on pr t in synthesi

I pota ium rrested he rt p fused t 7 85 kP (80 cm H_2O) nd vent icul pre su the ph nyl lal (ph) i corpo tion i t t l left ve tricul prot in in two hours w 1 50 _ 0 14 (mean _ S E M) µmol /g prot in and was i cre ed 1 91 _ 0 12 µmol /g prot when th pe f presso wa incr ased t 19 6 kPa (P 0 02)

When pot ium-arr ted heart we pe fused t 19 6 kP and the ent icula p su was re gul ed t 0 7 85 and 17 3 kP , ph i rporation two hours was 1 91 _ 0 12 2 10 _ 0 15 and 1 55 _ 0 14 µmol/g prot , espect ly These sult Ind cat h t the d cre in he protei yn he t highe entr cul p sure may be du t subendoca di l underpe fus on when the ven t cul pr ur pproaches th coronary pressure I be t g he t perfused 7 85 19 6 kPa the phe l corporation i two hou was 1 40 _ 0 04 2 02 _ 0 25 µmol/g prot

Und the periment l cond ti ns used th me- han cal wo k load doe not ffect the rat f pr t in ynth i myoca d um becau th yn hes t ll i be t g and rr ted heart hav ng th ame pe f p sure

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Has inen, I E and T kal T (Department f Med cal Biochemi try, U i rsity f Oul , Finland) TRANSMURAL GRADIENT IN PROTEIN SYNTHESIS OF LEFT VENTRICLE CAUSED BY ELEVATED DIASTOLIC PRESSURE IN ISOLATED PERFUSED HEART

T t dy the echan sm involved in the hype trophy and dil t tion f he l ft ent I Langende ff p fu ed rat he rt ed t d t rmin th ff t f chang i int ve t icu l pressure and mechan cal wo k on the prot i ynthesi f the l ft ent i l and t t ans mur l di t ibut

I potas ium ested he perf sed 19 6 *kPa (200 cm f) and itk t cul p es* su f ro 7 85 kPa the wa ansmural gradient i the phenyl lanin incorporation t t t l l ft entricul prot i Wh the en t icul pressu approached the coro ry pres sure (17 3 and 19 6 kP re pe t ly) the pro in ynthe i the subendocard l (ENDO) laye dimi i hed ignifi ly omp d th th d di subepi di l (EPI) l y rs f he same he rts, nd lso th t l pro in ynth the l ft ent i l wa redu ed ign f ca tly

I be ti g he rt be l ion f t pres- sure from 7 85 t 19 6 kP incr ased the phenyl lanine corpora on ig ifi an ly (T kal & Ha inen thes y ed ng) No t an mural g dien f amino id i corpe t on as b rved i the two group hen be d-dias li t t icul p su was levated from 4 40 t 5 50 kP and he rt pre wa kep t 7 85 kPa the amine id corpo tion educed i ENDO ompa ed ith the EPI f ame he rt ENDO f th ontrol he rt

The d f prot i ynth i i ENDO by lev ted di li p re l f on und pe f i f hi lay (ee Peuhku inen t l., the proceed g)

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Kuutinen, M T kal , T Hiltunen K and Ha i hen, I (Depa tme t f Medi l Bi chemi try, Uni versity f Oul Fi land) ON THE PRODUCTION OF AMMONIA IN RAT MYOCARDIUM

The puri ucleotid cycle (PNC) nd the gl tamat d hydrogen (GDH) ion the mo t lik ly cand d tes spon ibl for ammoni pro- duction i rat myocardium Th l i impo tan f the mechanisms i unknown I the pre ent tudy ammo i production was d t rmined in isol ted be ting potas ium re ted t hearts and h maximal tivities f GDH and the enzymes f PNC re me su ed i ca di and k l tal mu l

The deaminati f amino id i pe f ed h ts we 10 µmol /g d y wt/h i be ting and 3 µmol /g dry wt/h rrested h t

I t hea the maximal ti ti f PNC en ymes we 19 3 1 7 and 1 2 µmol /g dry wt/ i t 38 C for adenyl t deamina adenylsu i ynthetas od denyl suc ina , especti ly The orr spondi g l i k i t l mu l we 880, 2 1 and 3 3 µmol /g dry wt/mi Th ma mal ti ity f GDH pH 7 5 i the di tion f deami tion wa 3 8 µmol /g dry wt/ i i my cardium and 0 4 µmol /g dry wt/mi i k l tal muscl I th di ection f ami t on th ac ti iti we bout 5 time g t

The It i dicat hat myoc rdium th maximal capa ities f both thes ammoni pro duci g ystems much g t tha he he ed ammoni production The ituation i diff ent i kel t l mu l here PNC but not GDH h the capacity t ov he t l ra f ammoni produ i (Goodman & Lowe t l J B C. 252 5054 5060)

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Ask J,A Stene-Larsen G, and Hell K,B (Institute f Physi logy PRI Univ f Bergen Norway) THE CARDIAC β_2 ADRENOCEPTOR IN THE RAINBOW TROUT

We have tudied the adrenoceptor-medi ted responses in the heart f t leost with chi f ly adrenaline (A) in the sympathetic terminal i e the rainbow trout The hronotropic and Inotropi responses recorded isometrically in the spontaneously beating auri le *in vitro* were maximal t 17° and 14° respectively The force and frequency were potentiated by salbutamol (sal) at 2 14 C whil neither of the selective -adrenocepto agonists had any effect t this temperature interv l At 8 C the rder f gonist by ffinity wa Isoprenaline > A< sal> noradrenaline (NA) f the hronotropic and inotropic response The same rder was obtained f the maximal ffects f the gonists on the inotropic responses while for the frequency the rder was A NA> Isoprenaline sal The β_2 antagoni t H35 le 35/25 was the only one f the selective and β receptor blockers with ffects on the trout heart t 2 14 °C

Hence the trout heart i ocelleri ted via β_2-type f innerv ted ardiac adrenoceptor

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Wohlfart B and Edman K A P, (Department f Pharmacology, Un vers ty of Lund Sweden) AN ANALYSIS OF THE EFFECTS OF DIGITALIS ON THE EXCITATION-CONTRACTION MECHANISM OF MAMMALIAN MYOCARDIUM

Previous experiment have shown (Edman & Johannsson, J Physiol 1976 254 565-581) that mammalian myocardium produces optimum contractil response (OCR) 0,8 fter preceding stimulus and that the contractile strength thereaft lowly decays Peak force of OCR can be quantitatively predicted (Wohlfart Acta physiol scand 1979 in press) by the f llowing equation

OCR AP β F γ

In this equation AP and F denote action potential duration and peak force respectively f the nearest preceding contraction and β and γ are constants expressing the inotropic state The constant probably reflects the intensity f the transmembrane flow of activator calcium during the action potential and β the degree of reutilization of calcium released during the preceding contraction.

Isolated papillary muscles of the rabbit were paced to contract isomet ically at basal frequency of 0,67 Hz (37°C) AP and F were varied by introducing an extra beat and by varying the interval between the extra beat and the contraction preceding OCR Ouabain (0 3 µM) did not ffect the interval (0 8) required to attain OCR The glycoside potentiated OCR, caused marked ise f and less pronounced increase of β The positive inotropic effect of digitalis is therefore most likely ttributabl t an enhanced transport of cal ium into the cell during the action potential and to more fficient reutilization f the calcium that is released from the st res during excitation In high concentrations digitalis may also enhance the inflow of calcium through the cell membrane between action potentials This is indicated by the finding that 0,6 µM ouabain increases the rested-stat contraction i,e the contraction licited fter long (20 min) pause

Ek L Björkman J-A nd Carl son E (Pharmacol Dept AB Hässle Mölndal Sweden) CARDIAC INOTROPIC EFFECTS OF PRENALTEROL AND OUABAIN IN CONSCIOUS DOGS

Prenalterol an lly active β_1 selective adrenocepto gonist has been shown to elicit more marked timulation f cardia inotropy than chronotropy when compared to isoprenaline (Ca l son et l Naunyn-Schmiedebe g Arch Pharmacol 300 101 1977) Thi finding made it f interest to tudy the haemodynamic ffects f prenalterol (P) and ouabain (Ou) given lone or i combination Beagl dogs chronically i trumented with flow p obe round the ascending aorta and an i traarterial cathete were used ECG stroke volume (SV) rti flow accel ration (max dF/dt) and mean rterial blood pressur (MABP) were recorded Pr (45 nmol/kg) and Ou (50 nmol/kg) were given i and the effects were recorded t teady tat response The ffects xpressed as pe cent diff rence compared t saline control values were as f llows;

Substance	HR	MABP	SV	Ha	dF/dt	PQ interv
Pr	+1	0	20		34	-8
Ou	1	9	13		23	9
Pr Ou	1	3	24		59	-4

No rrhythmias were observed The data show that Pr ignificantly augments cardiac contractility with unchanged HR in the conscious dog The inotropic ffects f Ou and Pr seem t be additive which may imply that Pr i combination with digitali glycolycide may ff rd benefi ial ffects in the treatment f heart f il re

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Wollmer P , B Wohlfart and B Khan (Department f Pharmacology University f Lund Lund Sweden) EFFECTS OF 4-AMINOPYRIDINE ON ISOLATED PAPILLARY MUSCLES OF THE RABBIT

Micromole concentrations of 4-aminopyridine (4-AP) have been shown to greatly potentiate the release of transmitter at the neuromuscular junction There is les evidence for an increased release f transmitter in adrenergi nerves These effects have been attributed to an increase in action potential duration caused by an inhibition of the repolarizing K⁺ current Higher concentrations (1 mM) of 4-AP prolong the action potential and potentitat the isometric twitch in amphibian and mammalian skeletal muscl fibres probably by increasing the intracellula release f activator Ca^{++} In the present study the ffects f 4-AP were investigated in rabbit papillary muscles at 37°C. Membrane potentials were recorded imultaneously ith isomet ic force production 4-AP in relatively low concentration (0 01 mM) gave transient increase in force development which was counteracted by propranolol and therefore considered to be caused by noradrenalin release At higher concentrations 4-AP (in the presence of propranolol) had light positive inotropic ffect which reached an optimum (20-40% increase in peak force) at 0 1-0 2 mM. The inotropi ffect was associated with an increase in the ma imum rate f force development (dF/dt) and also ith slight increase in time to peak force and time t half relaxation The action potential progressively increased in duration over ide range of concentrations (0 01-2 mM) The results are explainable by assuming that 4-AP inhibits the repolarizing K current (cf above) The posit ve inotropic action may be due to prolongation and possibly also to an intensification f the Ca^{++} influx during the action potent al A similar mechanism has been proposed for the nerve terminal

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Sibgren C Nil son J -E , and Näwe U (Rese rch Lab at AB Leo l gberg Sweden) REDUCTION OF THE SIZE OF MYOCARDIAL INFARCTION BY HYALURONIDASE

Sever l subsets as h been l imed ffec tive in the tre tment f myocardial f rction both i pe iment l aimal and me En couraging cl ni l results have been obt ined by repeated int avenous i j ctions f hyaluronidase t the d se l l 500-1500 unit /k (P R Marak et l New Engl J Med 1977 296 898 903) When l ating possibl reduction f i f tion is i an it however gre t disadv tage th t k tari l is so heterogenous; standardi ed condit ons can be obt ined i pe iment l animal I th present study i f rction is t ind ced by coronary oc l i i t (N Bely et l Angi logy 1960 11 398-407) Hy l ron d se (Hy lase Leo) 1500-2000 IE/kg was inj eted i t venou l 2 4 18, 24 nd 42 hours ft induction lood samples were taken at 18 nd 48 hours f d t rmi ions f tot l LDH nd LDH 1 2

The LDH content eas were gnif cantly lowe i the hy l ronidase tre ted group compared t saline-inje ed entrol A d ff ence i the i f tion i between the tre ted and control t (18% smaller i the tre ted one) wa lso ident when we ghing th i f ctions fter 48 hou

I seems reasonabl t suggest th t the se f hy l ronidase ma be f ll cal l f reduction f the myocardi l f rct is

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Onarheim J and Tyssebotn I (Institut f Phy- i logy University f Berge Norway) EFFECT OF HIGH AMBIENT PRESSURE AND OXYGEN TENSION ON ORGAN BLOOD FLOW IN THE RAT

Although circul tory responses to pure oxygen t high pres ure have been reported we can find no data on rgan blood flow t high ambien p essure with normal O_2 tension (PO_2) The pr sent report summerizes blood flow measurement t high ambient pressure with normal and high PO_2 I mebumal anesthetized ts with core temperature mai tained t 37°C cardiac output and blood flow to major organs were measured with ^{85}Sr and ^{141}Ce labelled microspheres (15 µm) injected into the left ventri l In each rat measurements were performed t normal gas tmosphere/pressure (control) and in one f three expe imental ituations Group A: 5 ATA PO_2=0 2 ATA Group B 5 ATA PO_2=1 ATA, Group C 1 ATA, PO_2 1 ATA The inert gas was nitrogen Cardiac output heart rate and mean arterial pressure did no change in any group Renal blood flow f ll i all groups (p<0 05) In groups A and myocardial and cerebral blood flow increased by 30% (p<0 05) Diaphragmatic blood flow f ll in group C (p<0 001) Pulse pressure increased i groups A and B (p<0 05) due to el vated systo- ll pr ssure Respir tory ate fell in group B (p<0 02) Othe rgans (tomach intestine spleen liver lungs muscl) showed no change in blood flow

In conclusion In anesthetized rats high ambie t pres ur whether accompanied by high PO_2 not increase myocardial and cerebral blood flow High ambient pressure high PO_2 reduce renal blood flow The rise in pulse pressure uggests increased myocardial inotropy

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Husted S d Ned rg d O A (Depa tmen f Pharma l gy Aa hu Uni ity and Odens Un rsity Denma k) MAJOR ROLE OF PROSTAGLANDINS IN ADENOSINE TRIPHOSPHATE EVOKED INHIBITION OF VASCULAR ADRENERGIC NEUROEFFECTOR TRANSMISSION

The time ourse f h ffect f purine g ompound th con ra t l po f rabb is l ted pulmonary rt ry l d by lec ri al fi ld timul t n (150 mo ph pul ; 0 5 ms 3 Hz, 250 mA) wa ud d Ad no ne phosph t (ATP) β γ methyl e-ATP (APPCP t bl ATP an l gu), ADP AMP, deno and 2 hl re deno ne (1 10 7 3x10 4M) du d the neu ogen contra Th as lso th se f PGE_2 (3 10 10 3 10 7M) hal PGF_2 (10 10 4 10 7M) h d no ff I h f ATP and ADP th inh b tion l g l mi f dd tion than ft ll mi , wh l th ame f the th pu ne gl g Indome ha (5 10 6M) p tagl d yn h as nhib bell hed h d ff betwe th l nd ll nd inhibit used by ATP and ADP It had no ffect h APPCP voked h b t on Th nh bi ry ffec f ATP f l d ll xpo re wa gme t d by d l p (3 10 6M) pl de y cof rmycl (4x10 6M) nh b rs f d no apt h nd g respect ly Theophyl l ne (5 10 5M) had ff t Th b h can ed by ATP (10 4M) d re sed by he ext cellul Ca^2 t on (0 3 5 2 mM) and timul ti f equency (3 15 H)

I is co luded ha ATP lly us p esynap h bit on f ns l ma ly by an ADP med d h l i g l se f prost gl d (E ype) Th l h b t on i due ATP d me bol mainly den i which may po bly volve reduc i th ry f Ca the dre rgi

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Jsen D B and Ni lsen C K (Department f Pharmacology Leo Pharmaceutical Product Bal l rup Denmark) ADRENALIN INFUSION ENHANCES URINE KININ EXCRETION IN CONSCIOUS DOGS

In conscious t ined female mongr l dogs subpresso dose f adrenalin (0 5 mg/h) wa infused intravenously Aft one hour f control urin collection adren lin was infused f one hou and thi experimental period was followed by one hour post reatment urine collection Urine kallikrein was determined by TAMe-est ra se acti ity and urine kinin was measured by u oassay on isolated ra terus fter Ambe lit G 50 (H) adsorption and aceti acid (9M) lu ion (Acta physiol scand 1978 104 443) In ine experiment we obt ined the f llowing results

	Pre-control	Adrenalin infusion	Post cont l
Urine flow ml/h	11 3±1 2	13 3±2 7	9 7±1 0
Na -excr mmol/h	0 89 0 34	0 35±0 11	0 37±0 08
Kallikrein mEU/h	16 7±3 0	20 3±4 8	19 8±3 3
Kini -exc ng/h	41±9	191±29	56±12
ignificance			

The results show tha adrenali infusion en hances urine kini excretion whil t rine k llikrein remains unchanged

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Svensson, T H and Thorén P (Departments of Pharmacology and Physiology University of Göteborg, Sweden) LOCUS COERULEUS NEURONS: VAGALLY MEDIATED RESPONSE TO ALTERED BLOOD VOLUME

Several previous studies suggest that the pontine noradrenergi nucleus locus coeruleus (LC) may participate in maintenance of apprehensiveness and arousal as well as in the regulation or modulation of autonomi g cardiovascular func tion, including various reflexes Thus, electrical stimulation of the LC produces pressor response and recently afferent stimulation of the vagus nerve was found to cause inhibition of LC neuronal activity T further characterize the rol of LC neurons in autonomic function we have used singl cell recording techniques to study in the rat the LC response to physiologically relevant stimulus for cardiovascular reflexes, namely volume load The intravenous injection of 1-4 ml blood or dextran (Macrodex 10%) caused volume-dependent reduction in firing rate of LC neurons Withdrawal of corresponding amount of blood caused prompt return to base line activity Also bilateral vagotomy in the neck prevented this effect Thus, LC neurons respond reciprocally to moderate alterations of blood volume This effect i in all probability mediated via vagal afferents, presumably activated from atrial receptors

(Supported by the Swedish Medical Research Council proj Nos 4747 4764 and 0014)

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Lammintausta, R , Lamminta sta K , Pekkarinen, A and Seppälä H (Department f Pharmacology and Dermatology University of Turku Finland) RENIN IN HYDROCORTISONE HYPERTENSION OF RATS AND IN PREDNISONE THERAPY OF PATIENTS

Hyperten ion was induced in adult rats by hydrocortisone (20 mg/kg in drinking water or tail dipped daily in glycerol containing 2 5 mg/ml hydrocortisone) with 2 % saline as drinking wate 300 µl blood was sampled from tail vein t weeks 0 2 4 and monthly for adioimmunoassay of plasma renin activity (PRA) Blood pressure (BP) of 150 mmHg was reached in 4 8 week In 4 treatment series PRA rose regula ly 2 4 f ld in 2 3 weeks f t least 10 months (n=45 p<0 001) Propranol l (80 mg/kg) and metoprolol (100 mg/kg) in drinking water prevented hype tension below 125 mmHg, and PRA did not change from the pretreatment level (n=29) Drinking 2 % saline increased BP gradu lly up to 125 mmHg in 16-20 weeks whereas PRA decreased to half transiently f the first 8-12 weeks (n=27 p<0 01) DOCA (1 25 mg/kg) with line drinking induced hypertension up to 175 mmHg with tabl decrease in PRA to one tenth from the basal level (n=10 p<0 001)

Prednisone the py (30-60 mg daily) was tudied in 13 patients hospitalized for the treatment f dermatological diseases No other systemic drug therapy wa given BP wa found normal during therapy PRA increased by 46 % in 3 days and after 2 weeks therapy PRA was 120 % above the control level (n=9 p<0 05)

PRA in hydrocortisone-saline hypertension reflects more glucocorticoid than mineralocorticoid mechanism Thus in hypercortisolism f Cushing disease and in glucocorticoid therapy angiotensin an be contributing facto to hype ten ion

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kka J T Länsimies E Pitkänen M A , K O nd Valta R (Depa tments of Clin al Physiology and Lung Diseases Unive ity Centr l Hospital Kuopio Finl nd) RADIOCARDIOGRAPHIC STUDIES IN ALLERGIC ALVEOLITIS

Allergic lveolitis is rather common among the rural population of Eastern Finland Thi i learly correlated with the handling f moldy hay during winte season

46 objects with th mean age f 47 yrs (21-66 yrs) in acute or recovery phase of this diseas were tudied with common lung function measurements and with 113m-Indium radiocardiography RCG t est

RCG-pa ameters trok volume index SI l ft ventricular jection fraction LVEF pul monary blood volume index PBVI and pulmonary capillary pressur PCP were compared to the corre ponding figures f 46 control subjects The esults ar given in the f llowing tabl

	SI	LVEF	PBVI	PCP
mean	52	0 62	265	8 9
S D	12	0 11	60	1 6
number f pathological finding	3/46	6/46	19/46	11/46

A confidence limit for pathological findings i 95 % (control mean 2xS D)

The trok volume and pulmonary blood vol ume indices correlated ignificantly (p<0 01) with the arte ial blood oxygen partial pressur $ABPO_2$ (r=0 41 and r=0 47 respectively) but not with the diffusion capacity f lung DC There was nearly ignificant correlation (p<0 05) between the heart rate and $ABPO_2$ (r=-0 31)

The most promi ing RCG-index seems to be PBVI but further ex roise expe iments are equired to prove the usef lness f RCG-studies

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Ol n F, nd Ni lse L (Depa tm f Cli i l hy i logy Hvidø and R l Hospitals U i y f Copenha De ma k) DIGITAL ARTERIAL REO ING RESSURE DURING COOLING OF OUTDOOR WO KERS

He ex ame t f fi g ey toli blood p sure i h euff h iqu g i f rm i f arte i l pply nd t i l eop ing p be h h euff en ily be pe ed d i g fi g nd body co li g ing l i l eooli ff nd ooling blanket f ld wdy we i ig ed 59 outdoo wo k f mod body cooling ith finger p me d t 10 15 nd 6 C 35 pe sure h d g l ly wo k d ith h i d ll f h h d ompl int f hi fing i cold Thi wa ified by fing y li p essure 15 C 5 i 12 bj (digi l i l l) Th ib ti po ed sub J ow co pl i f hi fi g had ig ifi ly h gh eop sing pr 15 nd 5 ha h ne xpo ed ubj Th f g y li p lowe ed i the ih i xpo bjec Th be re l ear i li g ea th f be due to loc l co i p os es i b digi l rl Outdoo wo k wo ha ng ed ih i g eal had medi d ti i i g y toli p u 15 4 5 C i 91 nd 83 f the lwe 30°C (95 l we li i 66 nd 58)

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S.L.Niel e (Departme f Clini l hy i logy l Ho pi l Uni i y f Cop h g ea k) VENOUS PROTE CONCENTRAT ON ND CA PILLAR F LTRA ON DUR NG VENOU STASIS

Du i g ane ong i blood i pooled i the eul bed and pl ama ea tit filt ed th ough th pera bl wall Th p udy l i blood pooli g and tr eation on th h ma f rm flec ed i chang i f eara volume (tr i g age oh ique) ad vea pro i eo ti pled h lbo bef d ng ad aft i fl ion euff j et p inal lbow V eo ta i g l y i i f vo- l me f 10 mi wi h aa i i i l f i cr 2 an aiti l eo in pro eo on tr ti 3 on tan l l f 3 5 ai i 4 p aeuac d i ea i p i eonsea i lling venou p ar Th finding re pl i ed by rog en flow-filtr mod l h ae p ea t i fl et physi logi l mang i fl di ibu i and filtr i hi apo an ee id i g fil ion d nd p i bound eon itu in p aa

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Lundvall J and Hillman J (Institute of Physi logy nd Biophysics University f Lund Swede) FLUID TRANSFER FROM SKELETAL MUSCLE TO BLOOD DURING HEMORRHAGE

Mobilization f extra ascular fluid from sk le-tal muscle into the blood as result of decreased capillary pressure (P_c) is an important mechanism f plasma volume resto tion during bleeding The problem whether the decrease f P i passive and secondary t the fall f arte- l and venous pressure or if it i caused by sympatho- drenal active control f th pre to postcapillary resistance ratio (a/) i till unde debate This problem which has important functional implications was approached in the present cat experiments

Arteri l hemorrhagi hypotension as such did not influence P_c Thi co ld be ascribed to the fact th t the phenomenon f autoregul tion f P present i the control tate was preserved during bleeding demo t that t regulation of blood flow was impaired Further decrease f venous outflow pressure had little ffect on P_c The conclusion wa reached that the decrease f P_c and the attendant net transcapill ry fluid absorption was caused by sympatho-adrenal reflex es tting of a/ v This refle reset ing implied the a/ v as raised above the level that would be expected to result from the ut regul tory adjustments evoked by hemorragi hypotension per se yet a/ during hemorrhage decreased markedly below the level prevail ng i the normotensive control tate The sympatho-adrenal reflex adjustment f a/ v was mainly linked β-adrenergi dil to i fluence in v Such an ffect on can ff tively adjus P_c much l rge changes are required in i order caus imilar l rations f P

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Kauti inen M. Klinge E Lehtovirta P and Saarikoski, S (Department f Pharmacology Un versity of Helsinki II Cli ic of Obstetrics and Gynaecology Hel ink University Central Hospital and Tampere Central Hospital Finland) EFFECTS OF DRUGS ON PERFUSION AND INTRAUTERINE PRESSURE IN ISOLATED HUMAN UTERUS

Uteri (45-130 g) obtained from operat ons performed because of myomata were perfused through both uterine arteries by means of Multifi M 80 pump with 37 °C modified Tyrode solution The perfusion pressure was adjusted to 60-100 mmHg which yi lded perfusion rate of 10-40 l/ (T jo t l Acta Obst Gynaec Jap 1970 17 263) Drugs were i jected to the perfusion fluid nd thei effects on the perfusion and i trauterine pressure were recorded The plasma level f estradiol and progesterone were ssayed by RIA just before the operation The rati of rise i perfusion pressure (mmHg)/nmoles f drug njected was for various compounds follows 5-hydroxytryptam ne (5HT) 306 angi tensi (AT) 160 adrenaline (A) 30 noradrenaline (NA) 12 acetylcholine (ACh) 3 4 dopamine (DA) 0 06 and hi t amine (Hi) 0 02 I one times DA dose-dependently lowered the perfus on pressure AT i most cases produced bipha i ffect, i.e the contractions were preceded by transient rel xation The same ratios for the intrauterine pressure were AT 33 5HT 22 A 4 1 ACh 3.6 NA 1 6 Hi 0 2 and DA 0 03 I most uteri the responses t repeated i jections f drugs considerably declined withi 2-3 h The perfus on resistance wa higher i postmenopaus l teri Indomethac (27 9 µM) augmented the rise i perfusion pressure caused by A NA and DA Salbutamol nd isoprenaline dose-dependently lowered the i trauterine pres ure The trong ffects of 5HT and AT much resembled the res lts obtained t perfusion of isol ted human placentae (Klinge t l Ann Med exp Fenn 1966 44 369)

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Hell K B, and Bergh-Hanssen Q (In tit t f Physiol PAI Unive ity f Bergen Norway) PEPTIDE SENSITIVITY IN THE RAT PORTAL VEIN

The spontaneously active smooth muscles i the t portal vein have served mod l fo assessment f responses to vasoactive peptides l tive t the sympatheti eurotransmitter and appear highly sen itive t selected group f biologically active peptides

Five peptides contracted the venous smooth muscl in presence f cholinergic and β adrenergi blockade with the f llowing orde by ffinities angi ten i II> neuroten i brady kinin> substance P =phys laemin and by in- t insi acti ities angiotensin II> bradykini substance P neuroten in f the dose-dependen esponses The ffinities for these peptides were higher han that for no adrenaline I presence f lso 0 5 µM phent lamine the s-ponse t 0 15 µM angiotensi II wa twice that f 12 µM noradrena line Cocaine had no ffect on the ffinities or maximal ffect f the peptides while blocking the neuronal uptake f the sympa heti neurotransmitte

Two peptid oxytocin and vasopr in i hibi ed the spontaneous cont action in veins f femal and mal t f he Wi t t ain bot con acted those f Sprague-Dawley rats met-enkephalin somatostatin and numbe f ther peptides f ga tro-i tes inal origin were without ffect on he tension response i the rat portal vein

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Tl d 11 A R d S ds , E (Dep rtments of Physi l gy d Pathol gy I stit te f Medical Bi logy U iversity f Tromsø Norw y) THE INTIMAL SURFACE OF THE RABBIT AORTA

By ns f imec s two p e t s tting mat f 1 (11g 1 504) h y de ts of abbit o t s t pr ss res varying f om atm s ph 1 t ab yst lic We mo tored the di t i of the t by suring it di t wsi g a eptic l le system s d w blocked all b h t ri s by S phad sph s W fi d f ld e p s at on th i tim l s f t g t or less r te t at 11 deg e f di te io

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Sovijärvi A R A No set S , Hyvärinen J Fran il A , Graeff G and Lahtimäki M. (Departme t f Physiology Univer ity f Helsinki and Department f Electrical Engineering Tampe University of Technology Finland) EFFECT OF AIR IONISATION ON HEART RATE AND PERCEIVED EXERTION DURING EXERCISE

The influence f ai ioni tion on heart rate (HR) and ting f pe ceived exertion (RPE) during bicyol ex rcise was studied in 1ee healthy medical tudent andomly selected from the lass f 90 tudents The exercise test were pe f rmed both unde negative and positive ioni tion The study wa mad with double-blind ro over de ign The body surface xposed t ioni current was made large by reducing the l thing f the subject A ignifi ant over-all tendency to lowe HR and RPE values under negative ioni tion wa observed (P 0 01 ign test) The RPE values were ignifi antly lowe (P<0 01 paired t test and th Wilcoxon test) under negative than unde positive ioni tion t the maximal wo k load level Howeve HR values did not diff ignifi antly under negative and posi ti ionisation t any lative load level

The sult f this pilot tudy indicat that ioni composition of air an modify the RPE and pos ibly lso HR during xercise; negative ioni tion seems t be benefi i l compared with po itive ioni tion The mechanisms involved re becure but we ggest that negative ioni tion of ir may incr ase xidative metabol sm through gene tion f uperoxide radical (O_2) that i reduced t H_2O_2 by super xide dismutase

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d nd R (A s h i f op Denma)
CHANGES IN NTRA AND TRACE LULAR MUS TE CONTENT AN CYTE YTE CON NTRA ON MA UR INTE EXERC
i 11 d wa d h VY f 1 ma p um nd h f h pl sm i i g 1 od h g i i d tr 11 ar 1 Mg d 1 d al by me pe f rmed 3 he te f 3 i i icy 1 d y pe od M P (f ma 1 La d 1 pa h) nd f blood pl b be d 11 lar mi ed 1 di ibo i pa (jeg 9 9 Th 11 1 (1 ed f p (sea) nc ed fi ly me (1 D)) 0 ( ) mmol /1 f em 55 2) ma /1 d ro 0 5(08) 0 (Il ma 1 Mg/1 I La ii t ow mg g f g oora ed 1 ox ased f 3 3 15) 359 / d tr 1 f 3( ) 0(19) 1/1 0 g 1 om 9 8(g Is 5(5) mmol/100 g d ce dec ed f em) 1 1(13) mme /1 fi f d i Tr p h i Thi udy demo hang d 11 lyt eo ing ma h y eff ma i h i
Sj g ar O (9 9) Un Copenh g

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Bube N nd Secher, N H (Da i h N tional Sch ool t o f I f Cil Paraly and Medi al department P Bi pebj rg Ho pital Denma k) FORCE TIME INTEGRAL OF FAST AND SLOW TWITCH MUSCLE FIBERS AT ONSET OF EXERCISE

3 healthy y ung mal bje t performed 10 repeated ma mal voluntary ontra tions with finge muscl once very fifth d Bri k oom t t on were empha i ed I th mecanograms f t d low component could be parated F ro time i t gral (F TI) for th two compone t wa d t rmi d during the fi t 600 ms f h t t on

T ma ma mum for g for the f t nd the low ompo t 207 ± 6 9 d 590 52 (S E) ma pe ti ly Th fast ompone t had d rat on f 337 h 1 ms whil th low component could ma t i for 1 nd F TI as 9 3 ± 0 29 and 42 7 ± 0 61 N f the f t nd th low compone t re pecti ly The co respond g ma i um f ro were 51 7 ± 0 39 nd 102 8 6 N F TI f the f t ompo t had t 1 g t mpl t d fc 136 ± 3 7 ms During the 10 MVC re rdings the rel ti F TI f low componen di ea ed cordi g t the que ion 98 9 2 95 i 0 997 (di tignat th number f traction) F TI f th f t compone t did t chang (P 0 9)

I i gg ted that the f t and low component repre ent th me ogr ms f f t nd low i h mu l f bers re pecti ly T h f t t it h us l f b ha f i d th low t it h muscl fibe ha t ni contra tion patt i humans F tigue i repe ted ma imal lunta y contra tion may in l only the oni co ion lene t

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Sechar, N. H. (Department f Anaesthe i H r- le Hosp tal and August Krogh Institut Uni versity f Copenhagen Denmark) FORCE PATTERN DURING REPEATED MAXIMAL VOLUNTARY CONTRACTIONS 4 healthy mal subj ct performed repeated maximal voluntary isomet i contractions (MVC) with arm, hand finger mus les on very ten econds for 1ho t 1600 time in rde t re- cord the contraction pattern in f tigue I the f rc recordings two maxima were be rved one after 38 (17 88) the the aft 1 26 (80 2 4) (median value with 95% confidence limits) With repeated ffort f rce and EMG amplitud decreas d and the dura on f the force performance decreased from 2 3 t les than 6 with only one maximum in force ft pproximat ly 2 Changing from blindf lded t pe ye pe f rmance resulted in marked increase in f rce amplitude and re- appearance f two maxima contra tion patt rn The arly and l maxima are int rpreted to reflect the contra tion times f f (FT) and low twi ch (ST) muscl fibres re pective- ly Thus it i ugg t d that th FT fibre are deci ive for th t f rn f ension in MVC whil the ST fibre maintain force It i further uggested that ST fibre involvemen is decreasi g with repeat d maximal ff rt but that the ST mot unit can be partly reacti ted ith diverting ctiviti (Setchenovs phenomenon)

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Pet sen, S and Secher, N H (Medical depart ment P Bispebjerg Hospit l and Dani h National Association f Infantil Par lysis Hell rup Denma k) FORCE-TIME INTEGRAL OF FAST AND SLOW MUSCLE FIBRES IN FATIGUED MYASTHENIC PATIENTS

Repeated maximal voluntary cont actions were pe formed with finger muscles once every five second for eight minutes in four control sub- jects and in four myastheni patien Force- Time Integral (FTI) was determined during the first 1 25 f the contraction

FTI decreased by 30 % i the control subjects and by 65 % in the myasthenic patients In the mechanograms fast and low component could be separ ted Time t maximum f rce was 160 ms and 480 ms in the fast and low componen re- spectively The duration f the f st component was 320 ms and it did no hange with repeated ffort The low component had duration f several second In control ubject FTI f the f st and slow components was 6 6 and 75 Nxs respectively FTI f the fast component remained constant whil FTI f the low compo- nent decreased t 45 Nxs in f tigue In mya theni patient FTI of the fast component was 5 Nxs and did no hange with repeated ffort FTI f the low component decreased from 30 to 8 Nxs Prednison treatment had no ffect on FTI f the fast component but increased FTI f the low componen by 250-300 %

It i suggested that the f st and low com- ponent represent the mechanograms f the fast and low twitch muscle fibre respectively Thus fatigue in repeated maximal ffort may in- volve low twitch muscle fibre only In con- lusion myasthenia gravi is disease of ab- normal low twitch muscle fibre fatigue

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Ingemann-Hansen T Halkjær-Kristensen Hrage B Ch stensen N J, and Saltin, B (Lab Clin Physiol Exercise 2001 and Card ovas Lab Med Dept B Rigshospitalet Copenhagen 2nd Clin I tern Med Kommunehospital Arhus and August Krogh Institute Copenhagen Den mark) CENTRAL CIRCULATION AND PLASMA CATECHOL AMINE RESPONSES TO ONE LEGGED EXERCISE IN MAN INFLUENCE OF UNILATERAL MUSCULAR HYPOTROPHY

Exercise with hypotrophi muscles hows dif ferent cardiovascular and metaboli esponses as compared to work with normal muscles point ing to peripheral factor modulating exer esponses

Circul tory and metaboli responses were i vestig ted with the one-legged exercise model using 3 young heal hy subjects who f 5 weeks had one leg immobili ed in cas Each leg wa investigated separa ly fter the immobiliza tion period during graded ubmaximal and maxi mal bicycl work Maximal oxygen uptak VO_2max veraged 2 57 and 2 94 (l/min) respec ively for the weak and the control leg Hear te and cardiac output was linea ly related to VO_2 for both leg however the ela ion for the weak leg was posi ioned ignificantly (p<0) high er than for the control leg Noradrenaline in- creased from ve ge 0 25 2 16 ng/ml for the weak leg and to 3 43 for the con rol leg th the same curvelinear relat on ee gy expen- ditur (VO_2) ighly ign fican expone ial correlations wer found between mixed venous oxygen satur on and noradrenaline no diffe- ence wa obtained be ween the two legs(r=-0 94 n=21) Hypotrophic muscl induces gre t cen- tral irculation during dynami work than nor- mal muscl and hi reaction seems no ned ted by plasma techolamines

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Harr ,M H E (Department f Phy i l gy Universi ty f Kuopi Finland) PHYSICAL TRAINING UNDER THE INFLUENCE OF BETA BLOCKADE IN RATS

I ha been ugg ted hat many f the ad p- ve chang caused by physical t aining result from acti ion f the sympatheti rvou ys em during the rai period If thi hy- pothesi val d then physi l trai ing und he influenc f be blockad hould l ad t l defin t chang Thi ituation i very common mea who are dependen on long t rm drug therapy wi h be blockers and who lso are recommended includ physical tr ini g i he rehabilitat on program I thi tudy be combined ffect f phys cal trai i g and be blockad have been i ve tig ed i rat

Rat were tr ined by da ly running and swimm- ing rci for 4 t 8 week Both t ai ing me hod led t cardiac enl rgemen lowe ed resting heart increased heart-r t re ponse i oprenaline, increas d ac ivity f he oxida ive zyme in f t and low k l l mu l The tr ined t lso mai t ined high- l k empe ure when expos d 4 C However only swimmi g tr ined howed an incre d hype herni re pons t noradrenali smaller i the animal group ined und he influence f 10 g/kg f propr nol l

The presen resul emphasize the impor anc f he ympatheti nervou ys em i t i i g induced adaptive ha g If the sympathe i fluence i prevented by bet blockade lso the chang commonly con idered be ining -d pendent f ll t develop i pi f an equ l amoun f tr i ing

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Sellin, L C Libelius R Tågerud, S and Thesleff S (Department of Pharmacology University of Lund Sweden) MEMBRANE ALTERATIONS AFTER DENERVATION AND DURING REINNERVATION OF MAMMALIAN SKELETAL MUSCLE

Electrophysiological and radiotracer techniques were used to study the changes in membrane properties after denervation and during reinnervation f the extensor digitorum longus muscle f the mouse Denervation was performed ither by sectioning or crushing the nerve about 1 cm from the muscl After these procedures ll muscles showed f ll i resting potential (E_m) decrease in the rat f ise (dV/dt) f the action potenti l development f tetrodotoxin (TTX)-resistant action potentials and an increase in micropinocyti uptake of macromolecules.

Reinnervation of the nerve-crush muscles was indicated at 9 days by the appearance of miniature endplate potentials At this time there was small increase i E_m and decrease in the resistance to TTX Howeve dV/dt did not increase until fter 14 days f llowing crush despite the fact that about 90% f sampled fibers were blocked by TTX 14 days fter nerve crush *Upon reinnervation the rat f icropinocytosis* decreased with low time course reaching control values t about 21 days fte nerve crush time when the lectrophys logical parameters were at or near normal values I contrast muscles whose nerves had been sectioned showed all the signs of denervation

The results suggest that the rate f membrane turnove by micropinocytosis roughly parallels changes in membrane lectrical properties occurring fter denervation and during reinnervation The observed alterations in E_m and TTX resistance may be related to changes in specific membrane proteins whereas the slow recovery of dV/dt fter reinnervation may reflect the presence f other factors influencing membrane lect ical properties.

(Supported by Swedish Medical Research Council and NIH (USA) fellowship NS05935 (L C S)

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Husted S and Sjögren C (Institute f Pharmacology University f Aarhus Denmark and Research Lab f AB Leo Sweden) FIELD STIMULATION OF ISOLATED RABBIT URINARY BLADDER AND THE BIPHASIC NATURE OF ATP INDUCED CONTRACTION

Isolated rabbit detruso responded to ATP (10⁻⁴M) by an initi l phasi contraction which wa abolished by nifedipine (1 5x10⁻⁷M) an inhibito of Ca^{2} i flux and late tonic contraction superimposed by an increase in spontaneous acti ity of the preparation Indomethacin (5x10⁻⁴M) an inhibito f prostaglandin synthetase abolished thi l t phase f contraction Th non adrenergi non-cholinergi response (atropine 10⁻⁶M and guanethidine 3 4x10⁻⁶M present) to lectrical fi ld timul tion (5 sec trains f monophasi pulses 15 Hz 0 5 ms pulse duration upramax curr t) was mimicked by ATP i preparations pretreated with indo (5x10⁻⁶M) The direct contractil tension licited by $PGF_{2\alpha}$ PGE_2 o carbacholine was not influenced by indo neithe tropine (3x10⁻⁶-10⁻⁴M) no phentolamine (1 8 10⁻⁵M) tetrodotoxin (2 5x10⁻⁷g/ml) or theophylline (5x10⁻⁵ 10⁻⁴M) altered the ATP induced contraction Desensitization with ATP (10⁻⁴M) added repetitively to trips of rabbit detruso lectively depressed response to ATP without ffecting the response to noncholinergi non-adrenergi fi ld timulation while i the presence f indo (5x10⁻⁶M) both esponses were ignificantly reduced

It i concluded that both ATP and prostaglandins may play rol in the non-ad energi noncholinergi compone t of exitatory transmission in abbit urinary bladder ATP is eleased during lectrical nerve timul tion nd gives rise to synthesi and release f prostaglandins which i ease the sensiti ity of the preparation to i tramural non-adr non-choli nerve timulation

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Husted S (Inst C Pharm A h Denmark AB L Res lab N l ngb g nd Dep Cl Ph rm Lund Swed) MECHANISMS INVOLVED IN THE DIRECT CONTRACTILE EFFECT OF ATP AND OF FIELD STIMULATION OF ISOLATED RABBIT URINARY BLADDER AND EVIDENCE FOR PROSTAGLANDIN RELEASE MEDIATE BY ADP

I bbi inary bl dd b phas on et l ponse to ATP (1 4M) h be observed ADP (10⁻⁴M) i k d h l ton re ponse h b ould be lmost compl l bo lished by indomethaci (5 50x10⁻⁶M) l et t ADP the β T-m byl ne no t re f ATP (APPCP) 10⁻ 10⁻⁴M) whi h i r tab l ed t the dipheoph t l showed the ni l pha i response wh h l ghtly redu ed b ndomethacin but lly bol sh d b if di pine (3 10⁻⁷M) Adenosine (10⁻⁴ 1 5 10⁻³M) nd -chl road no ine (10⁻⁴M) rel ed he det so h 1 AMP (10⁻⁴M) nd dbr AMP (10⁻⁴M) how d no ffect I C free medium the response ATP wa abolished bef re hear high K nd C bachol PGE₂ (3 10⁻⁹M) nd PGF₂α (10⁻⁵M) ig ifi ly ugmen ed he pha pe se ATP he pres nc f dome ha but l lightly i un re d prepa ions l dome ha reduced he response f ld mul t 32% he ponse bei g compl l reest blished by add tion f PGE₂ or PGF₂α It oncluded h t th d ec on actil re po se f ATP on bbi d russo med d ms l h C i fl from he ac ll l pa nd he ten on de loped i augmented b pr landins rel sed from he ti ue when break down DP R l se f prost gl ndi du i g fi ld timul ti ma l more pro found med l t ry rel on he on ac il response li ited by he unknown un

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S V A Al k i H M (D p nt f Pharm l gy U i y f Ku pi Fi l d) THE EFFECT OF ADRENERGIC RECEPTOR BLOCKADE ON β AGONIST INDUCED RELAXATION OF RAT UTERUS IN VITRO

B d gi ti i ti i hibi l f d d gi i i p f lly i g p d p g imal Fi B d gi g i d if i p ll ylid i ip ns ll d ib l d i h p p i i h i h ph nyben ami (PBA) 0 g b i d f on Wi (120 150 g) p d i h di hyl stilbe l b h p ibi g i ff f b β-mi i d Th l i g i f h β-mime d h p y i hibi yi h l d d (10 2 g/l) ion Th β goni dd d t h b h 5 i h f re ACh Th i f PBA we 8 33 10 by whi h i lf d ff h did h g b ff f ACh Th I gi pA₂ l 5 i i b i f p li h p f h dru g i B pA₂-v l f 9 7 Ad ll lb t l ylid d ip nali g va A₂-v l f 9 4 9 0 8 1 d 7 8 p ive ly 100 l ip l d d g h an ft h re pre p h h d i h i p nali ff f ll h β g i p loop we ig ifi ly p i d by PBA p l ly the f lb an i I p₂-v l i d 9 8 I i lud d h ll h β g i h re d d p p ll on hev l a- d gi ff i y i

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Cherniack, N S, Euler C, von, Homma I, and Kao F.F. (Nobel Institute for Neurophysiology Karolinska Institutet, Stockholm, Sweden): AN ANIMAL MODEL OF CHEYNE-STOKES BREATHING.

The tendency for periodic breathing of the Cheyne Stokes type to occur was studied in anesthetized cats breathing either spontaneously with the aid of a servo-respirator governed continuously by the efferent phrenic nerve activity. Periodic breathing was brought to appearance by increasing controller gain. This could be done either by increasing the gain of the servo-respirator or efferently by lung deflation which increased the ventilatory response both to hypoxia and to hypercapnia. The propensity for periodic breathing was increased also by changing the chemical drive conditions so as to increase the relative influence of hypoxic drive either by increasing hypoxic drive and/or decreasing the central (CO_2, H^+) drive. The latter effect could be obtained by focal cooling at the ventral surface of the medulla. Less hypoxia was needed to produce periodic breathing at high controller gains than at low gains. Constant artificial respiration (zero gain) always abolished periodic breathing. Periodic breathing vanished when the relative contribution of the (CO_2, H^+) drive was increased by enriching the inspired gas with CO_2 or by letting the animal breath 100 % O_2.

The results are consistent with theoretical predictions for the occurrence of oscillations in the chemostatic regulator. They emphasize the importance of the (CO_2, H^+) drive in preserving ventilatory stability and suggest the possibility that instability in chemical controller plays a role in the genesis of Cheyne-Stokes breathing in humans.

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Hedner, T and Lundberg, P (Department of Pharmacology University of Göteborg, Sweden): NEONATAL ASPHYXIA AND CENTRAL MONOAMINE NEUROTRANSMISSION

Marked acute changes in the synthesis of the central monoamine neurotransmitters DA, NA and 5-HT are induced by hypoxia or anemia in neonatal rats. 6% hypoxia during 30 min in 4 days old rats markedly inhibited the formation of DOPA and 5-HTP in the brain after blockade of aromatic L-aminoacid decarboxylase with NSD 1015. In general this inhibition of monoamine synthesis was found in the hemisphere, striatum, midbrain and brainstem regions of the neonatal rats. In some brain regions shortlasting increases in DOPA and 5-HTP formation was found immediately after hypoxia.

In a separate study the acquisition of conditioned avoidance response (CAR) and the effects on monoamine neurotransmitter synthesis was investigated in 28 days old rats after neonatal oxygen deprivation (100% N_2 for 20 min or 6% O_2 94% for 4.5 h). The rats subjected to 6% O_2 94% N_2 for 4.5 h at 1 day of age were markedly inferior in the CAR acquisition than the control group. The animals subjected to 20 min neonatal anoxia did not differ in avoidance responding compared to control. An inhibition of tyrosine hydroxylase and tryptophan hydroxylase activity was found after neonatal exposure to 6% O_2 94% N_2 for 4.5 h while the rats exposed to 100% N_2 for 20 min did not differ from control. The precursor amino acids tyrosine and tryptophan and the endogenous monoamines DA NA and 5-HT did not differ from controls in any of the groups. It is suggested that the observed behavioural deficit after prolonged hypoxia may be due to an impaired development of central catecholamine mechanisms.

This project was supported by grant (no 2464) from the Swedish Medical Research Council

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Mattila M J and Muittari, A (Department of Pharmacology University of Helsinki and Pikonlinna Hospital Finland) BRONCHODILATOR EFFECT OF EPHEDRINE COMBINATIONS IN ASTHMATIC

T analyse th rong ronch d f f th combination f phedrine(E) eoph l line(T) hydroxyzine(H) ea l lin were conduc ed re ove in these re poma ve t l epremaline inha ea

Ora ingl d f E20 H 5 E20 xa epem 15 and pl be were g ven randoml 30 impa ient who sho ed morning peak xp re ry flow(PEF) rat 40-60% f ir lcul ted as incm Pl bo f iled modif PEF bu both active drug ombina i ns improved during 3 f th capsul intak E20 15 leng as ing previ ing rong rone ed l t and 3 hours

Othe 20 i pa i t having morn ng PEF rates as be ree i ed randoml E 30 E20 H10 p iphylline 200 E20 H1 nd p bo i l f rm I th se ume anee ve placebo impro ed PEF nd only igni f as drug f t we rded with h T130 E20 H10 combina ion 2 hou f ak hma i ou patien were rea ed b E25 T130 E25 0 E20 T 30 H nd place be rally i d f 4 y ach ea tively random rd Both (PEF home and work) and su j (rank ng) as demo rated he ff) f he rip comb nation ve p be Th combina tion two drug prev ou ea i) al- ly l p bl on rug ac ob-viously medi ied su jec abe ranking well indi idual symp oms and the se f isoprenal ne ressl Howeve the ipl combina ion used seems be balanced

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Meiby M, Aulie A, and Reite O B (Department f Physiology Veterinary College of Norway Oslo Norway) OXYGEN UPTAKE BY FOWL EMBRYO EFFECT OF OXYGEN AVAILABILITY

In the fowl embryo the O_2-uptak remains constant during the last week f incubation in spit f continued high growth rat One would therefore expect the embryo to experience progres ively hypoxi ituation The blood leaving the chorion-allantois is only about 73% saturated with oxygen in this pe iod Thus by increas ing the pO_2 in the environment more oxygen is made availabl to the embryo

The present study we undertaken to decide whethe embryos supplied ith mor oxygen increase their oxygen uptak The oxygen uptak f egg from domesti fowl was measured during incubation in ir i air fte 1 cm^2 opening into the ai space f the egg has been made and in pure O_2

Eggs incubated in air ith opened ir space exhibited 11% highe O_2 consumption than intact eggs on the last 4 days f incubation Intact egg incubated in pure O_2 increased their O_2 consumption with 21%

During the la t days f incubation the pO_2 f the gas in the air pa is about 100 mmHg When this pace i opened to ir the oxygena tion f blood passing the chorion- llantoi (th gas exchange) in this area i facilitated and the O_2 content f the blood ente ing the embryo should be increased I eggs incuba ed i pure O_2 all the blood ente ing the gas ex change i exposed to high O_2 Both ways f increasing the O_2 content f the blood ente i g the embryo resulted in higher O_2 uptak — the fowl embryo seem therefore able to adjust its metabolism to level compatibl with the av il ability of O_2 i their b

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Kauppinen Wal n K (Dep tme t f Biomedi l Sci es Uni rsity f T mpe Fi land):
REACTION OF RESPIRATORY AIRWAYS TO COLD EXPOSURE AT 10°C

I cold vi onment man ons rve and pro duc s be t by p iph l vaso t i tl i c as d t cholas et hi l g, t The tudy wa undertaken t xamine th influe ce f th r cti on the respi t ry functi

In preliminary xperiments 6 h althy adult femal s we xamined (i) l oom temperat re and (ii) in ld roo t 10°C The blood pre s re has t at ectal temp ratu and kin temper tu f f refsed and ha d re e-cord d With pa umota hograph th followi g re pi t ry functi e det rmi d FVC FEV_1 $MMEF_{25-75\%}$ PEF $\dot{V}_{max}$ 50 $\dot{V}_{max}$25, inspira t ry and expir tory olume d pi tory frequency

On th average ll respirat ry pa amet dimi i h d unde c ld xpo re th th p-t as f i pirat ry and xpir t ry olume d th FEV_1/FVC rati Th mean value in th old room pre d p rcenta f the i iti l warm room data re FVC 88% FEV_1 91% $MMEF_{25-75\%}$ 95% PEF 94% $\dot{V}_{max}$50 88% $\dot{V}_{max}$ 25 86% spirat ry frequency 86%; i p t ry volume 147% xpi tory volume 169% FEV_1/FVC % up 2 75 perc t un t Th h t t 107% y t lic blood pre re 106% dia toli bl d p 110% th tal t mpe t re 100%, th f head ki tempe t re 82% the ha d ki temperatur 80%

Ob ly the lung t as blood rvoi du ing cold xpo re Th lt l gge t pos ibility f fl on t t f rway du ing ld expos re

Gl EM(1949) J Phy l(London) 109 421 9

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Lagerspetz, K.Y H , Repo, T nd Saarinen K (Zoophysi logical Labo tory Department of Bi logy and Archipelago Research Institute Univer ity of Turku Finland)
Na AND K+ ACTIVATED ATP-ASES IN THE GILLS OF MUSSELS FROM FRESH AND BRACKISH WATER

The ite f active transport of ions are not known in bivalve molluscs However micro-somes from the gills f the freshwater mussel *Anodonta cygnea cellensis* show Mg^{2+}-dependent Na^+ K^+-stimulated well as Na^+ and K^+ stimulated ATPase activity (Lagerspetz K and Senius K Comp Biochem Physiol 1979 62B, 291) It is thus pos ible that the gill epi thelium is involved i the ion uptake of these animal I this study the ATPase activities wer studied in membrane preparations from gill f *Mytilus edulis* adapted to brackish wate with salinitie of 6 or 15 o/oo

Homogenate prepared from gill f *Mytilus* adapted to 6 o/oo showed monov lent cation timulated ATPase activity which wa about 1/5 of that found in *Anodonta* Na^+ and K -activa ted but ouabain-insensitive Depending on the assay condition the enzyme activity found in gill homogenate from *Mytilus* adapted to 15 o/oo wa 3-10 times higher A low Na^+ or K timulated ATPase activity was also observed Similar esults were obtained with microsomes

As the activities f membrane ATPases f the gills f *Mytilus* are ffected by environmental salinity they probably parti ipate in the ion regulation in this specie It can be suggested that in higher salinities more K^+ is exchanged for Na^+ by the high activity f the Na^+ and K^+ activated enzyme In the f eshwater mussel *Anodonta* the Na^+ K -activated enzyme is probably involved in the transport f ither cation from the dilute environment

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Le nonen, A (Departmen f Diagnost Rad ology Univers ty f Oul Fi land)
CINERADIO-GRAPHY IN THE EXAMINATION OF PHARYNGEAL LAR-YNGEAL AND TRACHEAL FUNCTION (Video)

Wi h ne adiography i i possibl t xam-ine even rap d function

Swallowing begins as an ac i func ion f the mouth and tongue and continue as reflex func ion of the pharynx The swallowed on as medium passes through th pharynx i abou half second and the upp oesophageal sphinc I open abou one se ond By func-ion can be seen the func on f he mouth in th con rac ion f the pharynx he uppe oesophageal sph te he losing f the larynx during swallowing

Larynge l movemen an be seen qui well even withou on rast medium bu the be me h-od is to mak he xamina on using on ras medium (ine laryngography) Pare i f he recurre nerve i he mos common fun tion l disorde i the larynx

Movement of the racheal ll an be seen ll i ineradiog aphy i hout on ras me-dium During f rced p ra ion and ough g tracheal lumen becomes narrowe than insp ration but normally no more han half f i inspiratory lumen In racheomalae rachea becomes narrowe than he f f l lumen I severe case t l ob ruc i an be see

I thi demons ration normal inerad ography f the pharynx laryn and rache and he most importan functional disorders are shown

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Bergqvist E and Öbrink K.J (Department of Physiolo-gy and Medical Biophysics University of Uppsala Sweden)
HISTAMINE AS A NORMAL MEDIATOR IN GASTRIC SECRETION

With the use of isolated glands from the gastric muco-sa of rabbit 1 new tool to analyze the stimulation procedure of the oxyntic lls is available The glands re pond to histamine and cholinergic drugs but not to gastrin Thi may be due to destruction of gastrin receptors but could also reflect the normal absence of such receptors In that case the action of gastrin in vivo hould depend on liberation of histamine which in turn would timulate the oxyntic cells In the glandular preparation the liberated histamine would be enormously diluted and would not reach concentrations high enough to stimulate the oxyntic cells

Histamine liberation has bee analyzed after stimula tion with pentagastrin and dose-effect curve was ob-tained with an ED50 at 1 nM pentagastrin The lowest effective concentrations of pentagastri were 10 to 100 pM The highest concentration of histamine libera-ted was below 0 1 uM which i the concentration barely detectable as giving secretory response in the gland Thus it has been possible t separate the two pe in proposed sequence namely the ffect of gastri to liberat histamine and the effect of histamine to timulate gastri secretion The conclusion is d at at least i the rabbit histamine is the final mediator for the action f gastrin on acid secretion

1 Berglindh T and Öbrink K J Acta Physiol Scand 96 150 158 1976

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Sundell G Sjöstrand S E , Olbe L.and Ryberg B. (Dept f Pharmacology AB Häs l Mölndal Sweden) PHARMACOLOGICAL INHIBITION OF GASTRIC ACID SECRETION BY A NEW MECHANISM.

A group of substituted benzimidaz les have been shown to inhibit gastric secretion They are more potent inhibitors of cid secr tion and have much longer dur tion f action than the histamine H_2-receptor antagonists

The inhibitory ffects on gastric acid secretion by substituted benzimidazoles and the H_2 receptor antagonist metiamide were compared in vivo and in vitro in various experimental models

In ivo tudi in the dog and in vitro studies on the isolated gastri mucosa of the guinea pig have confirmed that metiamide inhibits the histamine stimulated acid secr tion by competitive inte action The ubstituted benzimidaz les on the othe hand showed non competitive inhibitory action with dose-dependent reduction f the maximal acid response to hi tamine

Histamine stimulated secretion in the isolated gastri mucosal preparation was inhibited by both metiamide and benzimidazole Secretion stimulated by dibutyryl cycli AMP wa igni ficantly inhibited by the benzimidazoles whil metiamide had no ffect

In conclusion the mechanism f action f the substituted benzimidazol s in inhibiting acid secretion differ from that f the H_2-receptor antagonists

The substituted benzimidaz les seem to inter fere with secretory mechanisms periphe l to the H_2 receptor and peripheral t the cycli nucleotid step withi the pari tal cell

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Glise H , Abrahamsson, H and Lindahl B (Department f Physiology University of Göteborg, Sweden) SEGMENTAL DIFFERENCES IN REFLEX ADRENERGIC INHIBITION OF GASTRIC MOTILITY

Noc ceptive timuli applied anywhere in the gut, l it ate widespread adrenergi gastrointestinal inhibi tion i spinal reflex rche but littl is known to what extent such sympatho-sympatheti reflexes re segmentally different ted This problem was studied i anesthet zed vagotomized cats using as an ind cator gastri motility continuously recorded by volume method whil identical nocicept ve stimuli were appl ed to the wall of consecutive gastrointestinal segments

Nociceptive stimul tion of the small i testine or proximal colon (spl nchni supply) caused prompt and profound ga tri relaxation, whil similar stimuli to the di tal colon (lumbar colon c nerve supply) led to cons derably weaker gastr nhibitions The same d fference wa seen when instead identical lectric fferent stimul t ons were applied to thi nerve fila ments destined to the small intestine or proximal colon and to the di tal colon respectively The reflex le vations art ri l pressure were however equal whether proximal or di tal GI segments or fferent nerve f laments were stimulated All reflex responses were blocked by guanethidine or by pinal anesthesia whil they were unaffected by drenalectomy Thus noci ceptive timulat on of prox mal GI parts with splanchni nerve supply produces more pronounced reflex inhibi tions of gastric mot l ty than imila timul tions of di tal colon mai ly innervated by the lumba coloni nerves Th difference suggests considerabl segmental diff rentiation the spinal reflex centres for these inhibitory reflexes It cannot be ascribed to variat ons i GI ti sue sen t vity, f rst because the result were the same when mstead the fferent f bres were directly timul ted and, second,because reflex blood pressure responses were dent cal whether proximal or di tal segments were timul ted

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Flemström, G, (Department of Physiology and Medical Biophysics , University of Uppsala Sweden) STIMULATION OF HCO_3^- TRANSPORT IN ISOLATED BULL FROG DUODENUM BY SOME ULCEROPROTECTIVE PROSTAGLANDINS

Recent studies have suggested that the prostaglandins $F_{2\alpha}$ and 16 16 dimethyl E_2 protect the gastric mucosa from damag by intraluminal acid by stimulation of the active HCO_3^- transport in fundu and antrum Some other ulceroprotective prostaglandins (E_1 and I_2) seem to act by inhibition of fundic H^+ secretion Antiulcer effects of prostaglandins have however bee demonstrated also in the duodenum although no increase in pancreatic secretion of neutralizing HCO_3^- seem to occur Therefore it was of interest to study the effects of some prostaglandins on duodenal mucosal HCO_3^- transport

The latter is very probably an active transport originating from surface epithelial cell Isolated bullfrog duodenum preparation devoid of Brunner gland was used in the present study The bulb and adjacent part of proximal duodenum (exposed length 12 mm) was dissected free from muscle layers and mounted as tube between two glas tube connected to reservoir HCO_3^- transport into the luminal solution (gassed with 100 % O_2) was measured by titration with 5 mM HCl under automatic control from Radiometer pH-stat equipment The transmucosal lectri potential difference was recorded continuously

All preparations spontaneously alkalinized the luminal side Submucosal side administration of the prostaglandins E_2 (10^{-6} M) 16 16 dimethyl-E_2 (10^{-6} M) or $F_{2\alpha}$ (10^{-6} M) increased both the rate of alkalinization and the lectric potential difference Concentrations presented are those required for significant (p<0 05) effect withi 60 min after tart of exposure Maximal rat of HCO_3^- transport was higher with E_2 than with $F_{2\alpha}$ The stimulation of surface epithelial HCO_3^- transport may be one mechanism by which prostaglandins increase the ability of duodenal mucosa to resist acid

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Fjalland, B (Department of Bi logy Royal Danish School f Pharmacy, Copenhagen, Denmark) DO THERE EXIST SUBGROUPS OF HISTAMINE H_2 RECEPTORS IN THE GUINEA PIG ILEUM?

The antihype tensive gent lonidine has been shown to interf re with adrenergi well hi tamine H_2 receptors in various ti sues In the present tudy the pos ibl interference of lonidine with ther hi tamin rec ptors than the H_1 or H_2 wa tudied

The isolated guinea pig ileum coaxially timulated by supramaximal rectangular pulse f 1 ms duration 20-40 V t frequency of 0 1 Hz was used t t mod l

Clonidine concentr tion-dependently inhibi ted the twitche of the ileum with an IC_{50} of 7 0±0 9 nM The inhibition by lonidine was concentration-d pendently r versed by the H_2 recepto antagoni ts burimamid and imetidin IC_{50} for reversal was 1 6±0 3 and 71±17 μM re pectively whereas metiamid was without f fect in concentrations up to 40 μM The H_2 re ceptor agoni t dimap it also rever ed the inhi bitory ffect f lonidine, with an IC_{50} f 3 9 ±0 8 μM. Substanc which interf re with H_1 drenergi 5 HT or opiat rec ptors did not reve the eff t of lonidine After blockad f th H_1 recepto histamine its lf inhibited the EL-induced twitche an effect which wa reversed by burimamid and imetidine

The f t that hi tamine H_2 rec pto go ni ts and antagoni ts xhibit the same ffect on clonidine and in concentrations diff rent from those bserved i i the isolated tria may po ibly be xpl ined by the xi tence f hi tamine receptor in the ileum diff re t from the la ical" H_2 recepto

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Cassuto J, Jodal M, Lundgren O, and Tettle D (Department of Physi logy, Un vers ty of Goteborg Sweden): CHOLERA A NERVOUS DISEASE?

The profuse fluid los i cholera i according to current concepts the result of d rect cell la ction of the cholera tax on the i testinal epithelium Some observ tions, however indicate that lso other mechanisms may be involved Thus vasoactive i testinal polypeptide (VIP) that is known to be localized i nervous tissues is found i high amounts i the luminal contents during cholera The cholera exposed i testinal mucos lso exhibit a marked hyperemi an effect that als may be mediated via nerves (Biber 1973) It was therefore considered of interest to invest gate if nervous reflexes may be involved in the pathogenesis i cholera

The hypothesi was tested i two ways on cats where net water secretion was induced isolated small i testinal segments by cholera toxi First addi tion f the local anesthetic agent lidocaine to the luminal perfusate inhibited the cholera induced secretion and i half of the experiments it even produced a net fl id absorption from the lumen Changing the luminal perfusate back to solution without lidocaine restored the net fl id transport into the lumen I control experiments lidocaine luminal concentrations that inhibited the net cholera secretion was without effects on the net water uptake from normal intestinal segments Second, close i tra-arteri l administrat on of tetrodotoxin in doses that blocked splanchni sympatheti nerves also reversibly inhibited the cholera secretion i to the lumen

It is thus proposed that activation f an intramural nervous reflex may be one mechanism of importance i pl i ing the fluid loss from the small intestine i cholera Further, evidence also indicate that VIP may pos bly be involved in th s nervous reflex

Reference Biber B Acta physi l scand 1973 Suppl 401 1 31

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Youssef M and Suramo I (Department of Radi l gy University f Oul Finland) ULTRASONOGRAPHY IN PHYSIOLOGICAL AND PHARMACOLOGICAL EXAMINATIONS OF THE ABDOMEN

Ultrasonography i a non invasive technique ithout any hazards f ioni ing radi tion Theref re it is well suit d for physi logical and pharmacological examinations

The quipments and techniques have been developed rapidly during the last years Now it i possibl to achi ve with grayscal quipments anatomi inf rmation and imaging g f the tructure f the live pancreas splee and kidneys Real time systems are used t produc dynami inf rmation from vessels g: portal and caval veins aorta and bil duct tc

Images f diff rent physiological and pathological conditions are presented

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Bredin K., Nilsson G. Håkansson, R , and Sundler, F (Dep of Pharmacology Karolinska Institutet Stockholm, Dept of Physiology Faculty of Veterinary Medicine Swedish University f Agricultural Sciences Uppsala and Dept f Pharmacology and Histology University of Lund) TIME DEPENDENT INCREASE IN DUODENAL GASTRIN CONCENTRATION IN DOGS FOLLOWING ANTRECTOMY

The main gastrin producing tissue in dogs is the antral portion f the stomach Basal plasma gastrin level is no significantly lowered following antrectomy but markedly reduced after addi ional resection of the duodenal bulb (Sjödin and Nilsson 1975) Using RIA we have in pilot study demonstrated an increase in duodenal gastrin concentration following antrectomy (Nilsson and Bredin 1977) The ime course of this increase was tudied in 18 antrectomized dogs where the duodenum was removed 3 8-10 or 13-18 weeks after surgery The mean tissue concentration of gastrin in the proximal third of the duodenum increased by time being 73 ±15 3 76 ±33 0 and 238 ±174 4 ng/g tissue respectively compared to 13 ±7 0 ng/g tissue in unoperated controls Immunohistochemistry reveals an increased number of gastrin immunoreactive cells in the proximal part of the duodenum in antrectomized dogs In 3 dogs the mean gastrin concentration in proximal third f the duodenum 8 weeks following otal gastrectomy was found to be 46 5±26 2 ng/g tissue

The obtained results confirm tha when the main source of gastrin is removed there is ime dependent increase in duodenal gastrin concentration and in the number of G-cells which could be due to feedback mechanism Although there was no significant difference between the gastrin levels in proximal duodenum after antrectomy and otal gastrectomy this does no ral out our previous proposal that the acid producing part of the stomach exerts trophic influence on gastrin production ince the altera ion in acidity and composi ion f food reaching the duodenum following total gastrectomy might stimula gastrin production and decrease uch difference

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Kinnula V L, and Hassinen I (Departments f Phys logy and Medic l Biochemi try University f Oul Finland) EFFECTS OF HYPOXIA AND FASTING ON THE CYTOCHROME CONCENTRATIONS AND TURNOVER TIME OF INTESTINAL VILLOUS CELL MITOCHONDRIA IN THE RAT

Int tinal pithelial cell probably have the short st life-span f any mammalian cells whi h an be harve ted qua titatively and re theref re suit bl f the tudy of itochondrial biogene i provided that thei turnove time does not change

Epithelial villous ll were separated using isotoni mannitol solution and the mitochondri isol ted i the same medium Cytochrome were det rmined t liquid nitrogen temperature Mitochondrial protei turnove wa determined by i jecting 25 µCi f triti ted leucin intrape itone lly and observing the labelling f mitochondrial prot in up t 120 hrs Hypoxi was achieved in hypobari pressur hambe (40 8 kPa)

A paradoxic l ult in the cytochrome con ent tion wa obtained i ignifi ant in crease fter 48 hr in the hypoxic and f sting groups compared with the feeding control The half lif f the tot l mitochondri l proteins was 18 hrs in the feeding control and 32 and 36 hr i the hypoxi and fasting animal lcul ted from the prot in radioacti ity durin the first 48 hrs

Thi increase in cytochrome conten i probably due to the prolonged half lif f the itochondri llowing mo exten ive synthesis f mitochondrial component Thi indi t that the re lt f tudi on the synthesi f component f cell ganell may be distorted by hange in the life-span f the cell

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Ek B and Lundgren B (Research Laboratories AB Hässle Mölndal Sweden) CHARACTERIZATION OF β-ADRENOCEPTOR MEDIATED INHIBITION OF SPONTANEOUS CONTRACTILE ACTIVITY IN ISOLATED CAT COLON STRIPS

It is known that the spontaneous contractil activity in the cat colon strip is inhibited by α- and β-adrenocepto agonists In orde to characterise the β-adrenoceptors mediating the inhibition the ffect of increasing doses of three agonists isoprenaline prenalterol (β_1 selective) and terbutaline (β_2-selective) were studied ither alone or in combination with the antagonist metoprol l (β_1-selective) or IPS 339 (β_2-selective)

The spontaneous activity was characterized by recurrent bursts f contractions The i tegrated contractile activity was totally inhibited by isoprenaline (pD_2=8 3) The maximal ffect produced by prenalterol was 56% inhibition (pD_2=7 3 and by terbutaline 75% inhibition (pD_2 7 2) The terbutaline effect was blocked by IPS 339 but not by metoprolol while the prenalterol effect was blocked only by metoprol l The results indicate that both β1 and β2-adrenoceptors mediate inhibition f spontaneous activity in the cat colon

The remaining activity fter maximal doses f prenalterol was often modified into patt ern consi ting f ingl contractions of constant frequency Some additional experiments showed that tetrodotoxin modified the activity in imilar way prenalterol Thus the contractil activity apperently consists f two components one dominant neurogeni and myogenic component We suggest that β_1 adrenoceptor stimulation mainly ffects the neurogenic component and β_2 adrenocepto stimulation the myogeni component

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Puurunen, J (Departme f Pharmac l gy Uni ty f Oul Fi l nd) GASTRIC MUCOSAL BLOOD FLOW IN ETHANOL INDUCED MUCOSAL DAMAGE

The chang f g tri mu l blood flow oci ted with thanol induced damage f the gastri muc were tud ed in anes theti ed t

I g tion f the tomach th 10 20 nd 30 % thanol in 100 mM HCl pl 50 mM NaCl f 40 min damaged th gastri muco i concn trat on-depend t manne The g tri mu l ba wa b k the t flow f Na and K ons t the tomach and th back-d ffus on f H^+ ion t the ga tri mucos l rea d Furth rm re th re we l in the mu osa f rats tre ted th 20 and 30 % than l ^{14}C ami opy ine l rance f the gastri muc inc ea ed by tha l irrig t on in on en rat on-d pend t mann

Thi ty pe cent than l in sol t on on taini g 10 mM HCl 50 mM NaCl nd 90 mM ch l ne chl rid als broke the gastri muc l b rri sugg ted by outflow f Na nd K on f om th muc sa Howeve , becau the concn tration f d in lust li t as low the was l t l be k-d ffusion f H ions Only ome mucosal ul rs were b rved I the expe ime 30 % thanol d cr as d ^{14}C-aminopyrine learan of the g tri muc

The resul indicat th t than l its lf ha an nhib tory ffect ga tri mucos l blood flow Howev muc l blood flow i enhanced hen th g mucos i damaged by thanol in trongly d medium The inc ase f mu osal blood flow may be pro ective mechanism prevent g cumul t on f d withi th muc duri g damage

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Rähtinen, Juhani (Departmen of Rad l gy Uni rsity f Oul Fi land) HYPOTONIC ROENTGENOGRAPHY OF STOMACH DUODENUM AND COLON WITH GLUCAGON

Glucagon is rys alline polypep de with molecular weigh f 3485 and composed f 29 amino ac d re idu

Glucagon inhibi mo lity and ton i y of tomach small bowell and colon The hypotoni and hypomotility ff t comparabl propantheline brom de bu f shorter duration f action Glucagon has practically no ide-effec in small dose In doubl ontrast roen enography of tomach duodenum and colon relaxa on and inhibition f motility i impo an at good oating f barium to th mucosa Through the ffects f glucagon pasme can be diff ren i t d from rgani change and contrast medium doe pas oo early from tomach to duodenum Glucagon is an ffec tive and saf drug Radiographs of tomach duodenum and colon wi h doubl on ras me hod using glucagon are represe d

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Rarju, T (Department f Physi logy Unive ity f Oul Finland) DIETARY HABITS AND FACTORS INFLUENCING THEM IN SELECTED FINNISH GROUPS.

A dietary survey among 1175 people in selected groups aged 15 to 36 yr was arried out using the estimated food records method To tudy facto influencing food habit questionnaire we given to 73 f these

The sporting groups and the farmers ingested more foodstuff than others With the exception f these groups the energy intakes were at below the level for ligh physical wo k The main source f energy were milk ce eal and meat product Protein fats and carbohydrates covered 12 to 15 37 t 42 and 44 to 51 % f the intakes energy in the groups respectively

With the exception f itami A and ascorbic acid trient intake values were par ll l t energy intak The sedentary groups including students servicemen industrial and ffic workers ingested l t nut ient and included the greatest proportion (up to 90 %) f ubject not reaching the recommendation Iron vitamin A thiamine iboflavin and niacin di played the lowe t intak

Tast health and the price f food and the exampl se by mother f iend and f ther had the great influences on food habits Nut i ional inf rma ion came mainly from home news papers books and TV The subjects we aware f recommended practice The main f l in ea ing habits were the low energy intak sed by sedentary lif and atisfying the low need for energy by food wi h low nut ient density To improve Finni h ating habi incre sed importance should be given i political decision he l h poi t and peopl hould be encourag ed to us more vegeta ian non-refined diet

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V lkonen K and Piha, R S. (Department f B ochemistry U ive ity f Oulu Finland)
CHANGES IN LIVER HISTONES DURING BOVINE ONTOGENY

Chromati i composed f nucleosome in luding 200 base pair f DNA and an octet f pairs f each f the histones H4-H3 H2A-H2B whil the H1 histone ar bound to DNA on the outside of the nucleosomes Since hi tones ar thought to be involved in the control of templ te cti ity it ppeared of interest to study developmental changes in different histone fra tions during ontogeny

Live hi tone of calf embryos cal es and adult cows were analyzed Nuclei were isolated using sucrose gradient Total histones wer extr cted from pu ified nu lei with 0 2 M H_2SO_4 phenylmethylsulfonylfluo ide being used as proteolytic inhibito Analysi f the histones was performed by a sensiti polyacryl amide slab g l lectrophore i Five main hi tone types were present during the whol on togeny and changes in the lectrophor tic patterns f the hi tones especi lly f the lysine-ich histones wer found The H1 histone f embryos appeared as two component and that of the calves and of the adult cows three components According to the areas f the densi tometri records quantitative changes wer found in the main hi tone types during the bovine on togeny The rel ti amount of the H1 hi tone increased threef ld Change found in the H1 hi tone may reflect thei rol in the egu lation of the templ te acti ity and condensation of chromatin

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Eriksson,A -C, Eberg B ,Li R,M Sjöqui ,M. and Ulfendahl,H.R.(Department f Physi logy and Medical Biophy- Unive ity f Uppsal Sweden) VASCULAR RESISTANCE OF THE INTERLOBULAR ARTERY IN RAT KIDNEYS

The blood flow and the hydrost ti pressure patt rns within renal structures are determined by the flow re istance of differen enal vascul segments The aff rent and ff rent rt riol are generally accepted to be the main re istance segments However the interlobula art ries are small enough to off not neglect able re istance I order t quantitiea pressure drop long the int lobular artery the f llowing experiments were performed

Sprague-Dawley rats weighing about 300 g were anesthetized and prepared for micropuncture f the kidney With servo-system according to Wiederhj lm et l the hydrostati pre sure wi hin the renal structures were measured The ff ent rt ri les f glomeruli in the out cortex may enter the surface of the kidney and appea the tar vessels Oil i jected into the tar vessel act as blockade in the blood flow The hydros ti pressure i the blood column below the il blockage i he blood pressure at the end of an interlobular rtery

The top-flow pressures recorded amounted maximally to 80-85 mm Hg I number of recording pressure below three value were obtained The lowe value are explained by the fact he numbe f the fferent arteri les passing he renal surface give off branches to the peritubular capillary system

A ma hematical model describing the blood flow in the in lobular rtery and the ff rent art ri les made it possibl to isolate the influenc f vascula re is ance on the pressure drop along the interlobular art ry The calculations indicate as found in the rat the pressure drop f 40-50 mm Hg seems to be quite reliable

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M -l -C Ul (Department 1 Physi logy and Medical iophysic Univer ity f Uppsala Sweden) SIMULTANEOUS MEASUREMENTS OF FILTRATION RATE AND BLOOD FLOW OF SINGLE GLOMERULI IN RAT KIDNEYS

Experiments will be described where ingl nephron glomerula fil ration te SNGFR and ingl glomerula blood flow SGBF are measured The results will give contribu ion to the discussion abou he het rogene ty f nephron func ion

Ra were and Catheters we inserted int right common carotid rt ry femor l art ie femoral vein and he ureters Non-occ uded lamps were placed around the renal rtery and vein f both kidneys Radioac ve microspheres (15) 51-Cr-EDTA and 131 I-albumi were instantaneously jected int he aort and aft exac ly 10 secs the two renal lamps were activated and reference sampling f rterial blood we opped The deep-frozen kidneys we cut nto sections The samples wer analyzed i gamma-spectrome and SNGFR SGBF and medullary blood flow was alcul ed

	Control UC	MC	LC	F rosem de UC	MC	t LC
SGBF	198	134	83	99	98	76
SNGFR	2	15	19	13	8	8
SNFF	0 35	2	3	0 23	0 14	0 9
Blood flow IM	2 1 µl/mg issue			15 µl/mg		sue

V lues given in l/(glom 100 g rat)

The method i much les ime consuming than he Hanssen-echniques The microsphere techn que may overestimate blood flow i om cortex and underes imate in inner cortex some exten but the results of SNFF ad to sa i factory liabili y of the method The expe iments with f rosemide resulted de reas he h SGBF and SNGFR The decrease i SNGFR i not accou ed wh ch i seen i he low SNFF and i assumed depend on decreased net dri ing force across he g omerul membrane

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Jensen, P K and Steven K (Institute f Medical Physi logy Dept A The Panum In titute Uni versity f Copenhagen Denmark) INFLUENCE OF INTRATUBULAR PRESSURE ON PROXIMAL TUBULAR AND CAPILLARY DIAMETER IN THE RAT KIDNEY

The proximal tubula compliance was deter ined directly by measurements f tubula diamete and pres ure and indirectly using mathematical model f tubular fluid flow based on measurements f the hydrauli press gradient long the tubules under free flow conditions and during induced press re reduction t the end f the proximal convol ted tubul

The two independe t methods yi lded imilar value for compliance The change in tubular diamete was found to depend upon the intra tubular pressure the change in proximal tubu l diamete was ignificantly highe (p 0 001) when the intr tubular pressure wa reduced below normal (1 0 µm cmH_2O^{-1}) than when the pres sure we increased above the control value (0 4 µm cmH_2O^{-1}) A 50% reduction f 1 te proximal intratubular pressure re lted in 10% de creas i pre ure t the beginning f the tubule as calculated with the model

Also t ide tical value were mea ured in sodium pentobarbital and inactin anes thetized rats (p 0 5) The direct mea urement of tubul diamete in the control free flow ituation yi lded mean value f 23 0 µm (SE=0 7) and 22 3 µm (SE 1 2) which is in accordance with value f 23 µm calcul ted with the model

I tratubular pres ure changes resulted in inverse change i the diamete f the adjacent apillaries uggesting the the peritubul capillaries are distensibl tructures

The results show the nece ity f pressure control during tubular fluid collection

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Larsen Wibell t M and Wol er JM (Department f Physiology and Medical Biophysics Unive ity f Uppsal Sweden) VOLUME,HYDROSTATIC AND ONCOTIC PRESSURES WITHIN RENAL INTERSTITIUM.

I is well established that tubular fluid reabsorption will be governed by the hydrostati and coll id osmotic forces operating across the peritubula capil lary membrane The ignal will probably be mediated via the enal int rstitium.The present paper aims at the study of some tentatively important paramet rs vis the volume and the hydrostatic and oncoti pressure condi tions within the renal intersti ium

The interstitial and plasma volumes were measured as the distribution volume of 125-I labelled lbumin and 51-Cr-labelled EDTA analysed wi h indicator dilution technique with injection into the renal ar ry and sampling of renal venous blood Interstitial hydrostati pressure was obtained from direct punctures of enal subcapsular intersti ium.Coll id osmoti pre sure was analyzed from the protein concentration in subcapsular fluid sampled with small PVC-cathet rs introduced into the subcapsular space

During contr l conditions the inters itial space amounted to 48 ml/100 g rat increasing lightly 73 ml during 10% extracellul volume expansion In contrast, the hydrostati pressure increased teeply from 3.4 mm Hg to 12 1 mm Hg The corresponding colloid osmoti pressure decreased from 3 2 mm Hg to 1 0 mm Hg during 10% expansion

The compliance of the interstitium i thus comparatively low I is suggested tha the hange i i ters i tial driving forces hampers the removal f the tubular reabsorbate and thereby decrease he proximal tubular fluid reabsorption from lateral interspace

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Källs om G Ka lberg L, Mo lin B J Ol rs G & Wolgast M. (Departmen f Physiology and Medical ophysics Uni versity of Uppsala Uppsal Sweden) MEDULLARY CIRCULATION IN ACUTE RENAL FAILURE

Acute renal failure was induced by 45 min clamping f he renal art ry Thi produced classical acu renal failure with very small filtration an isotonuri polyuria and reduced potassium secretion.Micropuncture experimen showed tha the reduced tot l kidney filtration wa caused by either obstruc ion of the tubula lumen probably located to the medullary region or by gl merular schemia,with subsequent llapse f the nephron The total enal blood flow analysed wi h the microsphere echnique or the 86-Rb extraction method showed 60% decrease of the to al renal blood flow The medul lary blood flow was under normal condi ions,2 5 1/ml and g ti sue in the out medull decreasing successi vely owards 1 0 ml/ml in the inne medull During renal f ilure the blood flow in the inner tripe and the outer parts of the inne medull was dec ased 12 14% f the normal blood flows Thi insufficien medul lary blood perfusion caused an impaired func ion of he medullary tubular segments resulting in deficient urine concen tion ability.Microscop examination f kidney lices revealed congestion and stagnant blood in the reas with reduced blood flow predominantly the inner tripe f the outer zone of the medull

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Hilton, A Odlind B and Wibell L (Depart ments of Medical Pharmacology and Medicine University of Uppsal Sweden) PERITUBULAR UPTAKE OF INSULIN BY THE AVIAN KIDNEY

I an ttempt to tudy the renal pe itubular uptake f proteins we injected 125 I labelled porcine insulin human β_2-mi roglobulin (β_2-m) vasopressin (V) and β-endorphin (β-e) togethe with 51-C -EDTA into the renal portal system f hens (n=10 fo ach protein) prepared acco ding to the modified Sperbe technique The 125 I to 51-Cr acti ity rati in the injection mixture was approximately 1 The animals were killed 1 4 or 10 (30) min posti jection and the ipsi lateral (injected side) and contral te al (control ide) kidneys and the rgans wer removed The validity f the method was ve ified by calculating the corresponding 125-I to 51-Cr EDTA acti ity ratios fter i jection f 125 I Na-o-iodohippurate t 1/2 1 2 4 7 and 10 min post-injection The maximal ti (15 6 0 4 S D n=5) was found 1 min in the ipsilat kidney The corresponding value f the control kidney was 2 7 0 8 A ratio below 1 wa found in ll othe rgans At 1 mi there wa high rati fo insulin in the ipsilat kidney (4 4 2 0 S.D n=4) ignificantly greate than in the control kidney (2 0 0 2); t 10 min the ati was about 2 and approximately equal i the two kidneys For V and β-e there wa no increase i the ra tios for any f th kidneys compared to the injection solution Thi was true lso for β_2-m at 1 in whil the ratios at 10 and 30 min were around 2 equal for the two kidneys Conclusion The method used appears lid f studying renal pe i ubul r uptake f rgani an ions There seems to be ignificant peritubu lar uptake in the avian kidney f insuli bu not f the othe proteins studied

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Odl nd,B (Department of Medical Pharmacology,BMC Uni f Uppsal Sweden) RENAL TUBULAR SECRETION AND EFFECTS OF A NEW DIURETIC TIZOLEMIDE (HOE 740),AN ALKALINE SULPHONAMIDE

The modified Sperber techn que i the hen (Odlind,B Acta physi l scand 1978 103 404-412)wa used to study the rel tionsh p between renal tubular secretion and saluresi of 14-C tizolem de (Ti;3-9 ug/kg/ml) befor and during co-admi stration of inh bitors of orga i anion (novobioci ; 1 7 mg/kg/ml) or cat on (Deratine -mepiperphenidol-o l 25 mg/kg/ml) transport T and i hibitors were i fused nto the renal portal ystem vi leg vei The renal learances of 51-C -EDTA (C_{EDTA}) and 125-I-Na-o- iodohippurate (C_{HIPP}) were lso determined Result The tubul secret on efficiency of T i teady tate,a stimated by ts TTEF (true tubul excretion fraction) wa 60.2 3 7 % (S D ,n=7) The TTEF of T wa markedly reduced (to 6 9-12 8 % of pre sh t values) by Deratine,but it wa unaltered by novobioci The infus on f T i to one leg vei ,resulted an ipsil teral excess excretion of sodium of 45 2 ± 17 7 % (S D ;n=7;calcul ted percentage of the total sodium excretion).A corresponding value wa obtained for the ex ess excretion of chloride whil that of pota ium wa small (6 3 9 0 %) Deratine reduced these exces excretions f sodium and hloride roughly i proportion to the reduced TTEF values of T whil they were only slightly (g for sodium about 18 %) reduced by novobioci T had no effect on C_{EDTA} or C_{HIPP}.Novobioci reduced C_{HIPP} mark edly nd C_{EDTA} only lightly (to about 1/4 nd 3/4 of preinhib values resp) whil these were unaffected by Deratine Conclusion I the Sperber preparation T i acti vely secreted T to the urine by organi cation transport Th secret on of T seems to be importa t to its natriureti nd chloruretic effects I thi importa t respect T differs from cid sulphonamide diureti (thia ides) but resembles loop-d uretics Thus ignificant p rt of the saluretic effect of T i th hen appears to be evoked from the luminal ide of the nephron

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Hansen O Larsen, J A and Thomsen O Ø (In titut f Phy iology Unive ity of Aarhus Denmark) BIOLOGICAL ASPECTS OF VANADATE INTERACTION WITH THE SODIUM PUMP

Since the discovery of vanad te pote t inhibito f NaK ATPase consid abl interest ha been focused on it po ibl bi logic l importance Vanadium i chemically rel ted to phosphorus

I the present xpe iment some chara teri tics of vanadate-ATPase interaction were studi ed The specificity of (^{48}V)-binding we docu mented by (1) its facilitation by Mg^{2+} K (Kdiss 2-4 nM) and its inhibition by Na^+ ATP and Pi and by (ii) the identity f the total number f vana date nucleotide and ouabain binding ites i ctivated ATPase preparations from ox br i pig kidney out med lla and dogfi h rectal gland A method was developed f r d termination of vanadate in serum by measuring the displace ment f bound (^{48}V)vanadat to NaK ATPase with unl belled vanadat

Vanadate has been shown t act mo t potent diureti and natri tic i t (f 1) We inf med nadate in anaesthetized cat and were unabl to reproduce these ffects whereas ise i blood pressur 74% decrease i renal blood flow and second ry 90 100% reduction i urine flow we observed

It appears that in the cat vanadate act a potent vasoconstricto V soconstriction was evoked t serum vanadat concent tions f about 30 pM as determined by the above-me tioned method

(1) Balfour W E t 1 (1978) Natur 275 768

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Persson B E and Persson E (Department of Physiology & Medical Biophy ics Univer ity of Uppsala Sweden) MODULATION OF GLOMERULAR FILTRATION RATE VIA PLASMA PROTEIN CONCENTRATION

In poikilotherm lik Amphiuma means the urinary excretion rate changes on alteration of the extracellular volume t large extent by means of changes in GFR.In an ttempt to determine whether thi ffect is mediated by changes i the colloid osmotic pre sure of the blood experiments were performed in which an amphibian Ringer solution with or without 3g % bovine albumin was perfused into arteries supplying blood to 2 5 glomeruli On the kidney surface arteri supplying blood to several glomeruli are accessibl for micropunction The perfu ion rate used wa about 30 nl/min nephron.At the same tim a pressure recording cannula connected to servo-nulling pressure-recording system was used to puncture th fferent rteriol immediately before its division into the glomerul capillary network (GCP) It wa found that on perfusion f the artery with th sol tion without albumin GCP increased ignificantly (p < 0 001) from a control value f 9 8 ± 0 9 to 14 3 ± 0 8 cm H_2O (n=10) When amphibian Ringer solution containing 3g% lbumin was sed GCP decreased from 9 4 ± 0 5 to 4 3 ± 0 8 cm H_2O (n=7) From these result it ca be postulated that changes in colloid concentration might con titute mea by which singl glomerular blood flow, GCP and urinary excretion are altered in respons to changes in the degree of hydration

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ensen K E (Instit te f Medi l emi try University f Aarhus Denmark) GLOMERULAR FILTRABILITY OF FLEXIBLE POLYMERS IN THE RAT

The exc etion f different polyethylene gly col nd dextran f actions with narrow molecula weight range was compared to that f inu lin in th anaes heti ed ra The learanc f polyethylene glycol with molecular weight up to 4 000 was identical to that f inulin The mol cular weight di tribution f inulin in rat pl sma and urine was identical indicating no restri tion to ultr filtration f inulin in th t The ltrafiltrability f dextran declined from 100% towards 0% As Stokes adius w i re sed from 18A to 46A The decline f ultr filtrability f polyethylene glycol occurred t lower ngs f Stokes adii than f dextran Furthermore dextran ent Sephadex G-200 gel to higher extent than do polyethylene glycol of the same Stok adius The dependen ce f Stokes adius and ntrinsi iscosity on molecular weight indic t that polyethylene glycol i mor expanded in aqueous sol tion tha i dext Thi diff ence i att ibuted to chainbranching f the l tter polymer It i concluded that flexibl polymer probably because f terio hindrance permeat through wat filled po es to l se extent tha compact molecules f imilar Stoke adius Assuming that the apparent molecula adii f terio hind ance f flexibl polymers i glomerul membrane pores ar determined by thei penetr tion into Sephade G-200 columns cali b ted with globular pr teins the glomerul iering data f the present tudy sugges an ffective channel half-width for the t glomerulus of 55A

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Maxild, J, and Sheikh, M I (Institute f Medical Biochemi try University f Aarhus Denmark) ROLE OF N ON ACTIVE RENAL TRANSPORT OF PAH

The relation between metabolic energization f renal PAH accumul tion and Na^+ K^+ transport ha been tudied in rabbit cortical lices in the presenc of arious metaboli inhibitors and under anaerobi conditions 2 4-dinitrophenol CN nd F^- t low medium concentrations giving ise to light decline f oxygen consumption and ti sue ATP concentra tion caused reduction f PAH accumulation without ignificantly ffecting intracellular Na^+ K^+ concentr tions Inhibition f PAH accumulation under these conditions could be counteracted by addition f metaboli substr t Observ tions in the presenc f lincomycin ugg t that this compound causes a mod ate reduction f both PAH accumulation and Na^+ K^+ tr nspo t Studi on the t f ca rier-mediated PAH uptake showed that trans port slow unde ana robi conditions rela tive to that measured unde aerobi conditions in Na^+ depl ted lice I the l tter case the maximal accumul tion chieved was 1 55 0 16 suppo ting that Na^+ i indi pensabl f the ttainment of high accumul tion tios The uptake te f PAH under naerobi conditions could be inhibited by probenecid (1mM) and high concent tions of 2 4-dinitrophenol (5mM) Howeve the concentr tions f 2 4-dinitrophenol CN^- and F^- used f metabolic inhibition under aerobic conditions did not ignificantly ffect PAH transport i th absence f O_2 It is con luded that both Na -dependent and Na independent component exists in the energeti coupling f metabolism to ctive PAH transport

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Sheikh, M.I and Maxild J (Institut f Medical Biochemistry University of Aarhus Denmark) RENAL HANDLING OF PROBENECID AND ANALOGUES

The characteristics f renal transport of homologous series of hydrocarbon derivatives of sulfamyl benzoi acid were examined in co tical lices of rabbit kidney The length f the hydrocarbon substituent varied from C_1 to C_5 and the compounds differed from the previ ously examined probenecid series in having only one hydrocarbon substituent instead f two t the sulfamyl group Transport rat maximal accumulation and affinity f the monosubsti tuted compounds for transport were enhanced by an increase in the length f the hydrocarbon substituent In comparison with the probenecid series les binding by tissue constituents under anaerobic conditions was observed Competition experiments indicated that the monosubstituted compounds were transported by the same transport system as that f PAH Octa noate and fumarat exerted bipha i ffect on accumulation (i stimulation t low and inhibition t high metabolite concentrations) as is the case f other organic anions In comparison ith probenecid the monosubstituted compounds were more usceptibl to inhibition by PAH fumar te and octanoate The monosubstituted compounds were less hydrophobi than probenecid as evidenced by les extractability from an aqueous phase into an rgani liquid (1 2-dichloroethane) It is concluded that for the sulfamyl benzoi cid derivatives moderate hydrophobicity esults not only in higher affinity but lso in an enhanced turn over rate f the carrier system for organi anions

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Kopp U , Aurell M Svensson L and Ablad, B Dept f Pharmacology AB Hässle Mölnd l Sweden and Sahlgrenska Hospital Göteborg Sweden
EFFECTS OF PRENALTEROL A β 1 ADRENOCEPTOR AGONIST ON RENAL FUNCTION IN ANAESTHETIZED DOGS

Prenalterol has shown t be selective β-1 adrenoceptor agonist (Carlsson t l Naunyn Schmiedeberg Arch Pharmacol 300 101 1977) We have studied the ffects f prenalterol on kidney function in order to explor whether β-1 adrenoceptors are involved in the control f renin release renal blood flow and xcretory functions

Result Prenalterol 20 µg/kg i increased heart rate by 40 beats/min without changing mean arteri l pressure Prenalterol increased renin elease by 760% (p<0 005) the effect being independent of an intact renal innerv tion

Prenalterol tended to increase enal blood flow. Thi enal vasodil tor ffect was more marked in dogs undergoing water diuresi where prenalterol educed renal vascular resi tance by 14% (p< 0 001) in both innervated and denervated kidneys

Pr treatment with metoprolol 0 5 mg/kg β 1 adrenocepto antagonist reduced the chronotropic effect f prenalterol and abolished the ffects on renin release and renal blood flow

Whil glomerular filtration rate and sodium excretion were unaffected by prenalterol reduced urine volume(20-30%) and an increased urine osmolality (60%) was produced by the drug The l tte effects wer prevented by vagotomy and therefore probably due to an increased release f ADH reflex esponse secondary to the cardiostimulant ffect f prenalt rol(cf Sarier al J Cli Inve t 51 2613 1972)

Conclusion The results suggest the existence f β-1 adrenoceptors mediating renin release and dil tation of renal resistance vessels

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Veltonen H M and Eriksson L (Department of Physi logy College f Ve rinary Medi ine and Department f Veterinary Medi ine Universi y of Hel inki Finland) RETENTION OF ELECTROLYTES AND WATER DURING THE MATING SEASON IN REINDEER.

Seasonal environmental factor do no completely expl in the annual cycli alt tions of water and mineral balance in reindeer In this tudy periodi al changes in urinary sodium and potassium excretion were f llowed in reindeer during three years The animals we kept on low high protein di t wi h and without daily supplement f 10 g NaCl

On both high and low prot in diets with sal supplemen significan decrease f sodium excretion occurred during utumn and early inte Concurrently there was dec ease in potas um excr tion when the animal were on high prot diet with its inherently high potassium con tent However the plasma sodium and po assium concentrations did not change On low prot in diet without sal the urinary wate excretion was ignifica tly lowe in au umn and early wint than in sp ing Te there were no corresponding al tions in wa intak The pe iod f lectrolyt and wa re en ion coin ided with he mating se son i reindee and i likely to be sed by sex ro ds

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N ne nen M , Koskela, M , Leinonen Maila and Timisjarvi, J (Departments of Zoology Medical Microbiology and Physi logy Univer ity of Oul Finland) GROWTH AND SERUM PROTEINS IN THE REINDEER.

The reindeer in Finland is grazing in the forests and subarctic mountain areas undergoing an annual physiological regimen with highest metaboli demands occurring in the spring and early summer We i has been tudied the age and sex lated growth and electro- and immunolectrophoretical serum protein changes i 167 reindee The rapid weight gain is maximal (400 g/day) t an age of 4 to 8 weeks and the fi t winte tops the growth and so the growth can be depicted by 2 separ te polynomial functions

About 15 protein bands ar discernibl in the serum showing wider distribution in alpharegi on and IgG and also higher cathodi mobility f IgA than in human serum. The total serum protein is low the age of 20 days bu high in the adul hind and the difference i caused by globulin changes The mating season decreases the protein levels of stags as compared with those of hinds The gammaglobulin is high in the newborn calf but low 3 weeks l ter and highest in the adult hind.

I is sugge ed that the reindeer calf acquires passive immunity soon fte the birth by intestinal absorption of immunoglobulins and also that the endogenous immunoglobulin synthe is begins in the fourth week of lif and hence the pe iod just prior to thi forms critical stage partly expl ining the high mortality of the reindeer calf du ing the strenuous summer brought by drought and blood lling insects

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Brodin, E Gazelius B Olgart L. and Nilsson, G (Dept f Pharmacology Karolinska institutet Stockholm and Dept f Physiology Faculty f Veterinary Medicine Swedish University f Agricultural Sciences Uppsala)

SUBSTANCE P-LIKE IMMUNOREACTIVITY (SPLI) IN THE DENTAL PULP TISSUE CONCENTRATION, RELEASE AND DEGRADATION

The concentration of SPLI in dental pulp from cat dog and man determined by RIA was found to be comparabl or highe than levels found in other tissues outside the CNS The chromatographical behavior of SPLI on Sephadex G 25 in pulpal extract from ca was imilar to tha f synthetic bovine SP Incuba ion f cat canine teeth t 37°C for up t 4 hrs did not influenc pulpal SPLI content compared to levels in contralateral unincuba ed teeth Similar incubation f homogenized pulp tissue led rapid decrease in SPLI concentration in the homogenat Synthetic bovine SP and ^{125}I-tyr -SP were lso rapidly destructed by homogenized pulp tissue Repea ed an idromic stimula ion f the ipsilateral mandibular nerve (10V 15 Hz 3ms) prior to the incubation f the intact eth led to reduction f the SPLI content in pulps on the timulated side to 50-60% f the levels found in the contrala ral control ooth pulp The same type f nerve stimul ion lso increased the concentration of SPLI in pulpal supe fusates (20-42 pg/50 µl compared to 0-10 pg/50µl during control periods) and caused apid increase in pulp blood flow by 75% that was not blocked by propranolol (0 5 mg/kg) tropine 3 mg/kg) mepyramine(3 mg/kg) or imetidine (3 mg/kg)

The demonstrated lease of SPLI and increase i pulpal blood flow f llowing nerve stimulation together with potent vasodilata ory action of SP in this organ indi es hat SP may contribute to the development f he inflammatory responses associa ed with injury and pain in peripheral organs

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Anggård A Lundberg J M, Hökfelt T Nilsson G Fahrenkrug J and Said S (Depts of Oto-rhino-laryngology and Histology Karolinska Institute Stockholm, Dept of Physiology Swedish University of Agricultural Sciences Uppsala Sweden Dept of Clinical Chemistry Bispebjerg Hospital University of Copenhagen Denmark and Dept f Internal Medicine Dallas Texas USA)

INNERVATION OF CAT NASAL MUCOSA WITH SPECIAL REFERENCE TO RELATIONS BETWEEN PEPTIDERGIC AND CHOLINERGIC NEURONS

The origin and di tribution of nerves of cat nasal mucosa in normal and denervated animals were studied by immunohistochemistry using antibodies to substance P (SP) and VIP combined with staining for acetylcholinesterase (AChE) SP positive ll bodies were found in the trigeminal ganglia whil dense network of terminal-like structures were seen in the sphenopalatine ganglia (SG) around the principal ganglion cells In the nasal mucosa thin SP immunoreactive nerve fibres were seen in the subepithelial layer sometime penetrating into the epithelium VIP immunoreactivity was observed in the majority f SG cells and in dense ne works of nerve terminals in the nasal mucosa surrounding glands and blood vessel (mainly arterioles but also some sinusoids) AChE staining f the same section revealed very close overlap between AChE-rich and VIP positive cells in the SG Furthermore the distributions of AChE and VIP in the nasal mucosa were imilar After extirpation of the SG almost all VIP positive and AChE stained nerves disappeared in the nasal mucosa The presen data thus suggest possibl presence f VIP in some presumably cholinergic neurons (AChE i h) of cat innerva ing exocrine glands and blood vessels (See also Lundberg JM this volume) The use of AChE as marker for cholinergic neurons should however be interpreted with caution On the basis f the present findings hypothesis is advanced that local reflex arc exists involving sensory SP nerve endings in the nasal mucosa and in the sphenopalatine ganglion and vasomotor secretory (VIP-cholinergic) neurons

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Koivusalo F, Knip M Leppäluoto J and Ranieri, A (Department f Physiology Pedi t and Anatomy University f Oul Finland)

PANCREAS AS A SOURCE OF THYROTROPIN RELEASING-FACTOR (TRF)

We have previously shown that methanolic extracts f t gastrointe tinal tissues con tain immunoreactive TRF whi h in cation exchange and high-pressure liquid hromatography behaves as syntheti TRF The highe t concen tr tion we f und in the pancreas and this pape deals ith the igin f pancreatic TRF

Rat pancrea i i lets were isolated fter collagenase hydrolysis and submitted with the exoo ine part t tion exchange hromatography The f action co sponding to the lution f syntheti TRF were assayed i TRF radioimmunoassay The isl ts contained 0 21± 0 07 and the exocrine part 0 03-0 01 pg f TRF pe ug f protei

Triti ted TRF i jected in mice and whole body utoradiography reve led that the pancreas was unif rmly labelled The synthetic TRF was injected int mice no accumulation f exogenous TRF in the pancre could be found Our preliminary immunohistochemical tudies how that the FITC ma ke is localised in the out parts f the i ls afte incuba ion with an an i TRF serum

At the moment it seems that the pancr i TRF is product f the i let ll

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Mällinen M, Tuominen, M and Leppäluot J (Department of Physiology Univer ity f Oul Finland)

HIGH-PRESSURE LIQUID CHROMATOGRAPHIC EVIDENCE OF THE PRESENCE OF LRF IN RAT PANCREAS

Seve al bi logically active peptides are known t be located in neuroectodermally origi nating ti sues E g f hypothalamic hypophysiotropi peptides somat statin and TRF have been shown to occur in gastrointestinal ti ue In this report we show that LRF hypothalami hypophysiotropic peptide hormone is lso located in gastrointestinal tissues

Rat gastrointestinal ti ues were extracted with methanol (9 parts) The evapor ted resi due was ubmitted to CM-chromatography in 10 mM acetat f llowed by 200 mM ammonium The fractions corresponding to the elution f synthetic LRF were lyophilized and assayed by LRF radioimmunoassay Pancre tic xtracts howed the highest LRF level 1 pg/mg The f actions were then submitted t HPLC (MCH -column Varian with increasing acetonitril an luant) Pancre tic LRF immunoreacti ity was retained t 11 14 min t the ame time as synthetic triti ted LRF wa Our immunohistochemical tudies (to be publi hed) showed that pancre ti i let contained specific LRF positive material Hence we inf that LRF lso belongs t the biologically active peptides common t brain and intestine

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Leander S, Håkanson R, and Sundler F (Department of Pharmacology and Histology, University of Lund, Lund Sweden) NEUROPEPTIDES IN THE GUINEA PIG TAENIA COLI DISTRIBUTION AND EFFECTS

The many function f th dige tive t act seem t b controll d by compl int play f hormonal and neuronal ign l The int in i nerve have thei ll bodi in the myent i and submucos l pl Th myent i pl i considered to be en rned p domina tly ith smooth muscl acti ity Th di t ibution f b- stance P (SP) as tive intestinal p pt de (VIP) somatost ti d keph li wa t di d in th guin pig t eni coli by immunohi toche mical technique I dditi the ff t f these peptides on th mechani l acti ity f the smooth muscl wa tudied by conve ti nal *in vitro* methods Nerv di pl yi g VIP SP enk phalin immunore ti ity we nu i th was th mo l I th yent i pl us many nerves d ne ll bodies displ y d VIP immunorea ti ity whil SP d enk ph li nerv we few nd somat t ti ne ve re only ra ly ob ved

Ele t i fi ld timulation f t epi (10 $^{-6}$M) and gu neth din (5 10^{-6}M) bl k d p p t on result d in i iti l l ti f llowed by contr tion pe ce sati f timul t on VIP and SP ffect d th basal t ion f th t i well up addit on t th bath VIP on i g rel xation and SP cont ti Ma imal re pon se were obt i d t d se f 3 10 $^{-7}$M and 7 10 $^{-7}$M re pe tively The re pons we not ff ct d by t pine (10^{-6}M) phenoxybenzamine (10^{-7}) prop nol l (10^{-6} M) The re lt sug- ge t that VIP and SP pl y l in the regul tion f smooth muscl ti ty parh p eu o- t ansm tt

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[illegible] J M Schultzberg M, Hökfelt T Johansson L Sjöqvist A [illegible] L and Ramberg B (Depts of Histology and Surgery Karolinska Institute Stockholm and Medical Pharmacology University of Uppsala Sweden) ENKEPHALIN IN CAROTID BODY SIF-CELLS ADRENAL MEDULLA AND PHEOCHROMOCYTOMA

The occurrence f opioid peptides in various cate- cholamine (CA) containing chromaffin tissues of several species were investigated using immunohistochemistry with antibodies to α- and β-endorphin and to met and leu- enkephalin (ENK) No immunoreactivity (IR) was found in chromaffin cells with the two endorphin antisera. Type I cells of the carotid body in cat dog and monkey were strongly IR to met-ENK. SIF-cell in sympathetic ganglia of rat guinea pig and cat were also met-ENK positive In addition nerve terminals with ENK IR were seen around the SIF-cells Both met and leu-ENK IRs have been observed in adrenal medulla of all species so far studied e frog chicken rat guinea pig cow cat dog monkey and man Both noradrenaline and adrenaline cells seem to contain ENK ENK IR nerve terminals were also seen in the adrenal medulla of .g rat and guinea pig The ENK staining of these terminals disappeared after splanch- ni denervation In rat this denervation was accompanied by large increase of ENK-IR in the adrenal medullary cells reaching maximum after about one week. High spinal cord transection did however not cause any detectabl raise in ENK. CA levels of the adrenal gland were also normal one week ollowing denervation In bovine adrenal medulla the ENK IR had distinct granular appearance Met and leu-ENK were identified from bovine adrenal medullary extracts using Sephadex G-25 and re- versed phase HPLC columns combined with radioreceptor- and radioimmunoassays of the fractions A ratio of met/ /leu-ENK of 2 1 and 350 pmol met-ENK/g tissue were ob- tained for this gland. Human pheochromocytoma were also found to contain met-ENK IR in most cells In conclusion ENK seems to be present in various cells with high CA content

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Rosell S, and Rökaeus A (Department of Pharmacology Karolinska Institutet Sweden) THE EFFECT OF INGESTION OF NUTRIENTS ON CIRCULATING NEUROTENSIN LIKE IMMUNOREAC TIVITY (NTLI) IN THE HUMAN

Previous tudies in the human have shown the inges- tion of mixed meal causes marked rise in the concen- tration f NTLI in blood (Rosterman et al Lancet 1 78 I 785; Blackford et al Acta physiol scand 1978 104 ...) This study was designed to est if one or more of the principal nutrients in mixed meal may have any ma- jor ffect on the plasma NTLI Six healthy male volun- teers had by the oral route amino ac ds (30 ml as Vamin 3 itrum) glucose (30 as 300 ml Glucose ACO) or fa (11 g as 5 ml Intralipid Vitrum 300 mg ml $^{-1}$) Water was added to final volume of 500 ml following inges tion of Intralipid there was significant increase in the plasma NTLI concentration from pM (M SEM n=6) to peak value of 109 37 pM 90 min th re- turn f the plasma level by 90 min. The calculated mean release of NTLI increased from mM over 90 min to 1 3 2 mM over 180 min (n=6) Ingestion of isoca- loric amounts of Vamin and glucose di no change the plasma concentration of NTLI or the calculated mean re- lease significantl The results indica that f exert major influence on the concentra ion f NTLI in plasma I is suggested that neurotensin or subs ance derived from neurotensin is hormone invo ved in the post-pran- dial actions of f

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Gratt, D A Toro-Pedersen, C and Thorn N A (Institute f Medical Physiology C University f Copenhagen Denmark) ADENYLATE AND GUANYL- ATE CYCLASE ACTIVITIES IN SECRETOSOMES AND SE- CRETORY GRANULES FROM OX NEUROHYPOPHYSES

A secretosome fraction was prepared from fresh ox neurohypophyses by differenti l centri fugation and lysed by sonication Appea anc f cyclic nucleotides from their respective nucleo- ide triphosphate by radi immunoassay technique was used to determine adenylate (AC) and guanyl te cyclase (GC) acti ities At optimal Mg concentration (10 mM) AC activity was 80 1 14 3 (SE) (n=6) pmole cAMP/mg protein/minut whereas GC acti ity was 17 0 3 4 (n=8) pmoles cGMP/mg protein/minute t optimal Mn^{++} concen- tr tion (10 mM) More than 80% f the total GC activity in neurosecretosome was pelletabl 100,000 g for 20 minutes Rai ing the f ee Ca^{++} concentration from 10^{-} to 10^{-} or 10^{-3} M inhibited the AC acti ity Similar expe iments ith GC showed that 10^{-7} M Ca^{++} timul ted the GC acti ity considerably as compared to 10^{-7} or 10^{-4}M Ca^{++} Secretory granules purified on Percoll gradient and lmost free f Mg^{++}-Na K^{+} dependent ouabain sensi ive ATP activity had non-detectabl AC acti ity nd negligibl GC acti ity in contra t to the plasma membrane/ mitochondrial fraction From these sult we conclude that neurohypophyse l secretosomes tain adenylate and guanylate cyclase which are dependent upon the i ized tration and not localized to the granules

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Pohtola E nd Saarel S (Department of Zoology Zoophysi logi al Laboratory Universi ty f Oul Finland) EFFECTS OF BLOOD PRESSURE MANIPULATIONS ON SHIVERING IN THE PIGEON

Although often included i model of temper tur egul tion non-thermal influence on thermoregul tory effectors hav eceived littl experime tal inte est We hav tudied chang i shivering induced by blood pressure manipulations in the pigeon

Blood pressur (BP) from brachial artery and shivering (integr ted pectoral EMG) we measured from conscious pigeons placed in metaboli chamber t 10°C Noradrenaline (NA) was used to raise nd sodium nitroprusside (SNP) to reduce BP espectively An i.m i v i jection f NA inhibited shi ering when the increase in BP eached about 45 mmHg The inhibition had dynamic natur being more complete when BP changed pidly Shivering was later resto ed when BP started to decline When BP was nor malized by i.m or i v i jection f SNP trong burst f shivering sually f llowed However reduction of BP below normal had potent inhibitory affect on shivering which was not aboli hed until BP started to raise again

The results indic te that di placements in other physi logically regul ted ariables may hav profound ffects on the thermo egulatory contr l system Thi suggests hierarchial relation between body temperatur control nd other homeostati mechanisms

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Lahti K and Pyörnilä A (Department of Zoology Zoophy i logi l Labor tory Univer ity f Oulu Finl nd) THERMOREGULATORY EFFECTS OF CARBACHOL IN THE POSTERIOR HYPOTHALAMUS IN THE PIGEON

Thermoregulatory esponses to intrahypothalami injection f carbachol (CCh) were ecorded from unanaesthetized pigeons exposed to 20°C nd 38°C The drug (0 25 1 0 µg/µl) wa dmini t red i to the post ior hypothalamus which seems to be principelly concerned with the contr l f the esponse ag inst cold

At 20°C CCh i most case ither produced cessation of shive ing and led to hypothermia or produced bipha ic esponse light increase in body temperatur was f llowed by hypothermia I one pigeon the injections intensified shive ing nd an increase in body temper tur wa seen Th responses to CCh were not learly dose-depende t

Unde moderat heat load (38°C) CCh depressed the thermally induced pa ting nd caused hyperthermia A light increase i heart te was also ecorded Not even these ffects of CCh wer dose-dependent

The esponses to CCh in the posterior hypothalamus wer not as unambiguous in the t rior/medial pects (Pyörnilä A t l Neuropharmacol 16 737 741 1977 Neuropharmacol 1979 i pres) The esult suggest howeve that cholinergi mechanism located in the post rior hypothalamu is involved i temperatur regul tion in the pigeon Evidence for the exi tence f pathway mediating leo heat g in was obtained but the exact ites t whi h CCh produces diff ent esponses remai to be lucid ted

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l1 A (Department f Zoology Zoophysi cal Laboratory Unive ity of Oul land) FAILURE OF PALEOSTRIATAL LESIONS TO FECT THE APOMORPHINE INDUCED HYPOTHERMIA IN HE PIGEON

Apomorphine (intravenously or intr cereb oventricularly) evokes hypotherm i and certai te eotypies in the pigeon The precise loca tion of central dopaminergi receptors whi h mediate these ffects seems to be uncertain Since dopamine i found i g eat quantities in the paleost i tum in the pigeon brain experi ments wer ca ied out to d termine if this part of the b i is involved i tempe ture ffects of pomorphine

I tact birds were tested first The exper imen consisted f placing an unanesthetized nur trained pigeon in the metaboli hambe (ambient temper tu 20°C) and i jecting pomorphine (5 mg/kg i) emotely after abou 2 h adaptation i terv l The mea maximum chang (±S E) in ingpit temper ur was 1 8±0 22°C (n=6) 60-70 mi ft i jection

Bil t l electrolyti lesions (2 mA anodal current 20 sec) of the paleost i tum ugmentatum wer then produced i the same pigeons The body tempe tur fall induced by pomor phine (5 mg/kg i) 2 4 days fte surg ry was 1 5±0 12°C (±S E n=6) The differenc t nonlesioned bird i not ignificant ($p > 0.05$ t test)

A modul tory l in emper tu egulation f the mouse ha been post l ted f dopaminergi neurones f the audate nucleus (caudat corresponds t the avian p augmentatum) The presen results suggest that p augmentatum does not contribute t pomorphine hypothermi i the pigeon Supported by gr nt f om the Reikki and Hilma Honkanen Foundation

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Uldbjerg, H. (In titute f Physiology University f Aarhus Denmark) A VOLTAGE-CLAMP ANALYSIS OF THE EFFECT OF TETRACAINE ON AN IDENTIFIED SNAIL NEURONE

Afte impalement f an identified Helix Aspersa neurone (cell A Meech J Physl 1 249 (211 239) with two independe t screened microelectrodes voltage clamp tudies were made using conve tional techniques During depolari ing step the cell displ yed fast inward current and delayed outward current Ion substitution experiments showed that about 1/3 of the fa t inward current i carried by Na (I_{Na}) and 2/3 by Ca^{++} (I_{Ca}) The delayed outward current is carried by K^+ and ha two components one is voltage dependent ($I_{K(V)}$) and the the i tivated by Ca^{++} influx ($I_{K(Ca)}$) Supe fo ion with 0 1 mM tetracaine inhibited I_{Ca} (by 25%) more than I_{Na} (5%) 0 5 mM tetracaine inhibited $I_{K(Ca)}$ (79%) more than $I_{K(V)}$ (40%)

Tetracaine blocks Na -channel in many other ti ues t much small concentrations the used here (eg Skou Acta Pharm To 10 (281 291) In this preparation ll ioni channels investigated were inhibited by t tracaine but displ yed different sensitivities Thus the inhibition i not mer ly an un pecifi ffect f the relatively high conce tr tions used

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Jensen-Holm, J. (Department of Biophysics University of Copenhagen Denmark) CHOLINESTER DETECTION BASED ON AN IODINE IODIDE PRECIPITATION (IODINE-STARCH REACTION; TRACER TECHNIQUE; ULTRASTRUCTURAL LOCALISATION)

Acetylcholine i precipitated by treatment with strong solution of sodium polyiodide in water (NaI I_2 + NaI I where n in averag maximally is $1^{+}6$) One molecul of the ester is precipitated together with 8 10 atoms of iodine A blue colour i developed with soluble starch and read t 575 nm The molar extinction is up to 80 000 and less than 1nmol can be determined By us of $Na^{125}I$ the sensitivity markedly i increased (cou ting per formed in an Auto-Gamma spectrometer)

For electron microscopy the fixed and poly iodide treated specimens are washed with ether followed by an ether-Epon series and finally embedded in Epon The presumed cholinester-containing vesicles are heavily stained with an electron dense precipitate (acetylcholine-polyiodide?)

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Nordberg, A and Sundwall, A (Dept of Pharmacol Uni f Uppsala and Dept f Clin Pharmacol Karolinska Institute Huddinge Hospital Sweden) ASSESSMENT OF REGIONAL CHOLINERGIC NEURONAL ACTIVITY

Endogenous acetylcholine (ACh) ACh-turnover and high affinity choline uptake (H.A Ch uptake) have all been considered valuable markers for cholinergic neuronal activity The present communication compares the markers regarding regional differences in the brain f normal mice as well as in mice treated with oxotremorine (OT) and sodium pentobarbital A relationship is obtained between in vivo turnover values for five diff rent brain regions and Vmax values for in vitro H.A. Ch uptake

Pretreatment with OT and sodium pentobarbital produces specific regional changes in both turnove and H.A Ch uptake However both drugs produce shifts of the correlation lines due to uptak being less affected than turnove Atropine completely prevented the effects of OT regarding turnover and H A. Ch uptak

Separation of Ch metabolite in the synaptosomes evealed differences between the brain regions but the drug treatments had only minor effects on the proportion of ^3H-ACh formed This is major difference from what i obtained when Ch is given by intravenous injection when both drugs produce an extremely marked decrease in the ^3H-ACh formation Turnove thus seems more sensitive marker for cholinergic activity than H A Ch uptake The combination of the two methods has indicated dissociation between uptake of Ch and synthesis of ACh

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[illegible], A. Jahnsen and [illegible], D. (Institute of Medical Physiology Dept A the Panum Institute and Institute of Neurophysiology Copenhagen Denmark) INFLUENCE OF HYPOXIA ON HIPPOCAMPAL NERVE CELLS IN VITRO.

The extracellular concentration of K^+ in brain increases rapidly during cerebral ischemia ([illegible] A. Acta Physiol Scand 1978 102 324) whereas Na and Cl concentrations all ([illegible] A. unpublished) These ion shifts between intra- and extracellular space may be due to inhibition of active on transport or increased membrane permeability or both

Hippocampal lices from Guinea pig were maintained in chamber perfused with Ringer solution equilibrated with 95% O_2 and 5 CO_2 The input resistance of intracellularly recorded CA1 pyramidal cells as measured. The cells were synapticall activated by stimulating the Schaffer collateral and the minimal stimulus trength necessary to evoke action potentials was determined Hypoxia was produced by perfusing the chamber with Ringer solution equilibrated with 95 N_2 and 5% CO_2 The oxygen tension of the chamber medium was monitored.

In response to hypoxi the input resistance decreased and the threshold or generating action potentials increased until no action potentials could be evoked During these changes the cells initially hyperpolarized 5-10 mV and then depolarized. Upon reperfusion th [illegible] started to repolarize [illegible] Ringer [illegible] the [illegible] could not be maintained. but intracellular recording could not be [illegible] after hypoxi had Othe cells in the same lice impaled after [illegible] and three normal membrane potential input resistance and threshold.

This tudy demonstr tes that hypoxia induces decrease of input resistance The initi f [illegible] probably due to an increase of K^+ conductance as shown by the hyperpolarization and [illegible] The rapi on shif observed [illegible] conductance increase The rapi [illegible] ity can be xplained by increased membrane permeability

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Olsson K.A. and Landgren S (Dept of Physiology University of Umeå, Sweden) FACILITATION AND INHIBITION OF JAW REFLEXES EVOKED BY ELECTRICAL STIMULATION OF THE CEREBRAL CORTEX IN THE CAT

The effects of conditioning lectrical timulation (1 10 anodal pulses 0 5 ms duration 400 600 Hz) of the cerebral cortex on the monosynaptic jaw closing and the di-synapti jaw opening reflexes were studied i cats anaesthetized with chloralose

Alternating phases of facilitation and inhibition f the reflexes were observed The reflexes were i iti lly inhibited or facilitated with l tencies as short as 3 5 ms which may indicate di or tri ynapti paths from the cerebral cortex to the trigeminal motoneurones

The most effi ient effects originated i rea 3a of the coronal gyrus Large ffects were lso evoked from rea 3b whereas areas 4γ 3a 6aβ and 43 were less effi ient. The observ tions indicate that the l rgest ffects on the jaw reflexes were evoked from the primary projection reas of the oral cavity nd the face (the projections f the ipsil teral lvmo) nerves and the nerve t the contral teral maxillary whi kers)

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Hansen, A. (Institute of Medical Physiology Dept A, The Panum Institute Copenhagen Denmark) BRAIN EXTRACELLULAR IONS IN ISCHEMIA AND SPREADING DEPRESSION

The extracellular concentr tion of potassium K_e in brain increases i ischemia and spreading depression (SD) (Hansen A.J Act Physiol Scand. 1977 99 412) is unknown howeve to wha exten the concentration of othe ions are affected

Spontaneously breathing ats were anaesthetized with pentobarbital and bur holes placed in the parietal and frontal bones Microelectrodes sensitive to K^+ Na Cl Ca^{++} and H^+ were inserted i the extracellular space of parietal cortex pH was measured with glass lectrode while other ion concentr tions were measured with liquid-exchange lectrodes SD was elicited by briefly stabbing the frontal cortex with needle and was monitored by the transient neg tive change f the DC-potential Following the passage of SD ion concentrations were allowed to recover before the heart as arrested by injecting saturated $MgCl_2$ i

Normal ion concentr tions were (mM) K_e 3 1 Na_e 153 Cl_e 130 Ca_e 1 1 and pH 7 4 During SD the concentrations transiently changed to (mM) K_e 38 Na_e 56 Cl_e 90 Ca_e 0 05 and pH 7 2 The vari tion of Ca_e and pH was of longer duration than the change of the other ions At the onse of ischemi pH immedi ely began to fall as K_e lowly increased whil the othe ions did not change significantl Afte 1 2 min f ischemi when the DC potential became negative K_e increased rapidl and Na_e Cl_e and Ca_e showed sudden decrease Extreme lues af ter 10 min of ischemia were (mM) K_e 80 Na_e 44 Cl_e 68 Ca_e 0 05 and pH 7 0

Thus brai cells are unable to maintain ion gradient the onset of SD and after few min of ischemia

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A i1 L H J I k en R nd Ah n L (Di i i f Ph rm l gy Dep ne t Pharma y U i i y f H l i ki Fi land): EFFECTS OF ACUTE MORPHINE ADMINISTRATION ON CATECHOLAMINES IN DIFFERENT PARTS OF THE RAT BRAIN

Th ff f morphi 1 the t f -m hyl p- yre i (MT 200 mg/kg i p 2 h) i d ed b 1 d pami (DA) d na 1i (NA) depl tion udi d i mal Wi t t g di g i i nd the morphi d se q i d Morphi dmi i t ed t diff t i gl d (E p A 10 g/kg; Exp B 5 10 20 40 g/kg) nd he ve d apit t d ft diff p iod (E p A 2 1/6 2 1/2 3 4 6 h; Exp B 2 1/2 4 8 h) DA nd NA i ve mea ed i fl b i p li bi p (LIM) p i t (STR) hemi ph (HEM) di ph lon (DIE) nd 1 we b i tem (LBS)

Ma imum 1 ti f h αMT induced NA d pl tion ed i E p A 4 h f mor-phi (10 mg/kg) ad i i i i LIM nd STR The b 1 t ly high t 1 i g ff 1 E p B w hi d ith th small t d g (5 mg/kg) t 2 1/2 h i LIM (f om 48% t 66% P 0 001) nd STR (fro 38% t 54% P 0 001) Th ff till i id t 4 h (P 0 05) i h d withi 8 h With 10 nd 20 g/kg f rphi 1 i d the ame l vel p i ti g th gh 4 h d di pp i g ithi 8 h well Morphi (10 g/kg) se med have ligh 1 i g ff t NA d pl tion i DIE I LBS di i d se p longed 1 ti d 1 dy f 5 mg/kg HEM f il d t h y ig ifi re i

I i 1 d d th t 1 ady 5 mg/kg f rphi ffi t ms imally 1 t αMT ind d DA d NA d pl ti (the f rma i LIM nd STR the 1 t i LBS)

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Hellevuo K Attila L M J Andersson K and Kiianmaa K (Res Labs State Al Monopoly; Dept Pharm Uni f Helsinki Helsinki Finland and Dept Hi tol Karolinska Inst Stockholm Sweden) ON THE ROLE OF CENTRAL CATECHOLAMINE NEURONS IN ETHANOL INTOXICATION

To tudy the rol f central catecholamine neurons in thanol intoxic tion the brai c techolamine level f rats were lowered in three diff rent experiments In the first study newborn rats were tre ted with 6-OHDA (3 100 mg/kg 24 h interval) f selective reduction f brain noradrenaline I the the two experiments dult ts were sed 6-OHDA was injected bilaterally lose to ither the ascending dopamine (8 μg/4 1) or noradrenaline (16 g/4 1) pathway to lower the b in dopamine noradrenaline levels respecti ly Endog nous no adrenaline in the neonatally 6-OHDA treated nimals was selectively reduced by 100 % i the cerebr l cortex 35 % in the midbrain and increased by 117 % in the pons-medull I adult ts 6-OHDA caused marked degener tion of the dopamine noradrenaline pathways nea which it was injected Fo tudying motor co-rdination fte thanol admini tr tion (2 g/kg i p) the tilting plane te t wa sed Neonatal depl tion f b ai no adrenaline caused the rats to be ignificantly mo ffected by thanol than the control when me sured t the ag f fou months Thi was lso evident f rats depl ted f brai noradrenaline as adults I contrast thanol impair d the performance of the dopamine lesioned ts ignifi tly les than that f the control These finding suggest rol for the central catecholamine neurons i the in oxicating ffect f thanol

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Wikberg J E S nd Axelsson K.L (Department of Pharmacology Linköping Uni ersity S-581 85 Linköping Sweden) AN EFFICIENT METHOD FOR STUDYING NEUROTRANSMITTER RELEASE IN VITRO BY THE RADIOTRACER TECHNIQUES

A system has been constructed which llows the simul taneous measurement of neurotransmitter release from four independent ti sue preparations by utilizing the radiochemical methods for measuring ^{3}H-noradrenaline and ^{3}H-acetylcholine (Wikberg 1977 Acta physiol scand 101 302 317) The device consists of fou perspex holders to which the preparations can be mounted Incubation and washing of the pecimens i performed by immersing the holders i scintill tion vials contai ing the pproprì te medium. For thi purpos 48 i ls re kept i a stai les teel holder i a termostated water bath -sequenti l washing is performed by transferring the perspex holders i to new ial After the incubations the acti ity i the washing solutions can be measured direct ly fter addition of scintillation cocktail to the vi l Oxygenation of the medium i performed through polyethylene tubings mounted to each one of the perspex holders Two platinum lectrodes re lso mounted to each holder allowing lectrical field stimulation of the preparations I order to timul te the four preparations imultaneously using only one physi logical timulator cheap lectroni device (physi logical stimulator dis tributor; PSD) has been invented The PSD consi ts of four reed relays which re appropriately timed by integrated TTL ircuitry i order to di tribute the pulses from one Grass timul tor sequenti lly to four output channel By thi pproach timul tion of ll four preparations can be performed t all desired pulse durations and pulse frequencies without the need f three extra timulators The whol system ha been tested and found to work properly both for measuring ^{3}H-noradrenaline release from guinea pig vas deferens and ^{3}H-acetyl choline release from guinea pig ileum

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Stjärne L nd Alberts P (Department of Physiology I Karolinska Institutet Stockholm Sweden) DEPENDENCE OF 3H-NORADRENALINE SECRETION FROM ADRENERGIC NERVES OF GUINEA-PIG VAS DEFERENS ON STIMULATION FREQUENCY CALCIUM AND α-ADRENOCEPTOR- AND PGE2-MEDIATED CONTROL

In guinea-pig isol ted as deferens pre-labelled with 3H ( )-noradrenaline (NA) repetitive electrical nerve stimulation caused progres ive ise in secretion f 3H-NA per hock (At) Facilitation was compl t fter 15 to 300 shocks depending on the frequency By extrapolation the singl shock val for At was esti mated High calcium concentration in the medium, increased frequency f timulation or α-blockade with 1 µM phentolamine (PA) ised At t all levels f facilitation but did not lter the magnitude of facilitation (tio f fully facilitated At / singl shock At remained 15) 1 µM noradrenaline howeve selectively depressed ingl shock At or short train At Kinetic analysis showed that ise in train length or in frequency f stimul tion (from 0 25 to 5 Hz) enhanced both the affinity for external cal ium (K_m) and the maximum secr tory velocity t infinit cal ium concentration (V_{max}) Blockade of α-adrenoceptors with 1 µM PA raised and 10 nM PGE2 depressed V_{max} but moder tely if t all depressed K_m The enhancing ffect f PA on At we markedly educed upon stimulation with high pota ium (80 mM) however

(Supported by the Swedish Medical Research Council nd Karolin ka Instit tets Fonder)

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Meyer-Rochow V B (Department f Bi logical Science University of Waikato Hamilton New Zealand) THE EFFECT OF LIGHT AND TEMPERATURE ON PHOTORECEPTOR CELL ULTRASTRUCTURE IN THE ANTARCTIC CRUSTACEAN *ORCHOMENE PLEBS*

Temperature is well known to affect the re pons characteristics and photo-regeneration f visual receptor cell ; temperature induced ultrastructural changes f photoreceptive cells, howeve have not been demonstrated bef re

By using the Antarcti amphipod *Orchomene* which inhabit wat f constant temper ture f 1 8 C it was possibl to show that an exposure f 7 hours t temperature f 10 C caus d th visual membranes t break up and lose their tructural int grity The rhabdoms were grot equely deformed and the creening pigments f these heat-stres d animals ven when dark-adapt d were in the light adapted position Animals that were returned t wat f 0 C and examined 7 hours later showed remark bl recovery the vi ual membranes had re-organised microvill patt rns had reformed and the rhabdom tructure had fully re generated The screening pigments too had already commenced to migrat int the dark dapted position

Thes experiment which were ccompanied by ERG recordings and f tty acid analyse f head-lipids show that light and temperature cause separate but intricat ly related ff cts that mus not be studied in isolation from ach the

This work as completed during the 8th expedition f the University f Waikato Antarcti Re Unit and funded by grants from the U G C (New Zealand)

The support f the Antarctic Division (D S I R) and the U S Navy i cknowledged

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Jørgen en, F. (Phys l g cal I t , Odens Un ersity Denmark) THE INFLUENCE OF CALCIUM AND D 600 ON THE MECHANOSENSITIVITY OF THE HAIR CELLS OF THE LATERAL LINE SYSTEM OF NECTURUS

The rôl f cal ium in th an duce pro ce f the hai ll th l er l line y tem f *Necturus* has be vest ga d by xam ini g th ffect f change in th chem cal gradient f Ca ro th ep l pa f th hai cell on th mechano ens tivity f thes cell Furth rmore th ffe f D 600 (Ca antagoni t) on the mechanos ns tivity as examined

Th mechan ens vi y f th hai cell was det rmined as the probab l ty f occurren f aff rent spikes from the ch rel ve th phas f mechan cal st mul on (POS) Th results f chang ng Ca_o thereby chang g the chemi l gr dien ac th ha b ring part f th cell howed th POS was no redu d from 10 M antly reduc d hen Ca_o ducti f Ca_o reduced t 5 10 M Fu th ducti f Ca_o in ensitive me POS l ving th hai cells ins nsitive 9 10 Th xpe chanical t mul t on Ca pH 7 1 (T -Cl) and ri gnt were conduct d ed on EGTA Ca below 10 M as ob betw en POS and At pH 6 6 th rel on h p value f Ca_o Ca_o wa hif d towards h gh sel on con l When D 600 dded reduc d t con entra ing Ca_o 10 M POS became reduc d 55 g/l POS ti high than 15 mg/l and i i i l value Th f was reduced t 10% f th reversibl f ct f D 600 unaff ed by l rge

Th fi d g hat POS i acro the chang in the chemi l grad en the ir mec f pig l memb ane may que on the ff t f C as ch rge carr How ve f th D 600 ugg ts th Ca i tran duc proces

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Hulliger M, Nordh, E Thelin A,-E. and Vallbo A.B (Department f Physi logy, University of Umeå, Sweden) RESPONSES OF MECHANOSENSITIVE AFFERENT UNITS IN THE HUMAN HAND TO MOVEMENTS.

The response to direct touch of the low-threshold mechanosensitive units innervating the glabrous ski f the hand is well described, but littl i known about their behaviour during active movements The purpos of the present work wa to tudy whether these fferents are activated by voluntary movements

T thi end activity from singl units i the median nerve we recorded with microelectrodes during natural finge movements Precautions were taken to void incidental activation of the units by direct touch

All four types of fferents (i rapidly adapting RA- and PC-units and slowly adapting SA I and SA II units) nd majority of the recorded units were found to be ctivated during these movements The responses during the dynamic phases f the movements were much more frequent than during tati conditions even for the SA units.

Since the low-threshold mechanosensitive units do respond to voluntary movements it was concluded that they may be of importance not only for tactil mechanisms but for kinesthesi and motor performance as well

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Mäkelä J and Putkonen P T S (Department of Physiology University of Helsinki Finland)
ALPHA ADRENOCEPTOR AGONISTS VERSUS ANTAGONISTS AND PARADOXICAL SLEEP CONTROL IN THE RAT

The α-adrenoceptor stimulant clonidine (CL) appears to be a universally effective suppressor of paradoxical sleep (PS) in vertebrates from birds to man. In the cat this effect is antagonized by several α-blockers, excluding selective postsynaptic antagonists thymoxamine and phenoxybenzamine (Putkonen P et al. In Pharmacology of the States of Alertness, Pergamon Press. In print). Both phentolamine (PH) and yohimbine (YO) effectively antagonized CL, and PH even increased PS when given alone.

To determine the effect of α-blockers on the PS-suppressing effect of CL in rats, 12 hour day-time polygraphic recordings were used. CL (100µg/kg) inhibited PS for ca 8 h and decreased 12 h values of PS from 8 2±2 4% (SD) to 3 9±1 2% (p<0 01 N=6 t test). Pretreatment with neither PH (10 mg/kg X PS=3 1±1 7%) nor with YO (1 mg/kg X PS 4 7±2 1%) could significantly antagonize CL. With YO however there was tendency to increased PS in the last 4 h of the records whereas with PH the corresponding PS% was even below that of CL alone.

This is in sharp contrast to our earlier results in cats, especially with respect to PH, which is an effective antagonist to the PS-suppressing action of CL even in chicks (Putkonen P and Helsinki M. This meeting). Thus in spite of seemingly universal inhibitory action of CL on PS our results suggest notable species-differences in the pharmacological profile of central catecholamine receptors which can influence the control of PS.

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Alihanka J, and Ha an J (Department of Physiology University of Turku Finland)
TEMPORAL DISTRIBUTION OF BODY MOVEMENTS DURING NORMAL WHOLE NIGHT SLEEP

Temporal distribution of body movements during whole night sleep recording in ten 20 25 year old male students were studied during 20 nights. EEG EOG EKG and submental EMG were recorded. The Static Charge Sensitive Bed (SCSB) method was used for recording of the durations of the movements (Alihanka J and K Vaahtoranta Electroenceph clin Neurophysiol 1979 in press). The total number of movements during one night was between 80 and 200. The movements were divided into four groups according to their duration 5s 5 10s 10 15s and 15. The interval between all consecutive movements and between the movements within each group we measured separately. Interval histograms were made of each night and of the whole material with class interval of 20 sec. The Kolmogorov test was used to determine the differences between the measured movement intervals and random interval created with Poisson process. It was found that the movement intervals were not randomly distributed either if all movements of the night were considered or within any of the groups. It was also found that in the group of movements 5 intervals 1 min in the group of 5-10 intervals between 5 20 min and in the group of 15s intervals longer than 20 min were overrepresented respectively. Short movements appeared often in series while the interval between long movements were often long. The recording of the different movement patterns provides useful method for studying cyclic physiological processes involving the motor system during sleep.

Grants The Juho Vainio Foundation

E and Johansson G (Institute of Physiology University of 00170 Helsinki 17 Finland)
REMODULATION OF THE MEAL PATTERN OF THE RAT DURING RAPID EYE MOVEMENT SLEEP DEPRIVATION

Changes in the feeding schedule seem to affect the amount of following rapid eye movement sleep (REMS) in the rat (Mouret and Bobillier Intern J Neuroscience 2 265 1971). Correspondingly the amount of REMS within period of 12 hours has been shown to predict food intake during the following 12 hours in the cat (Siegel Physiol Behav 15 399 1975).

In this work, study has been made of the relationship between feeding behaviour and REMS deprivation in the rat.

Twenty-four hour cycles of food intake were continuously recorded in male rats on 12/12 light dark (LD) schedule during baseline conditions and REMS deprivation.

The baseline food intake was similar to that reported by Armstrong et al (Physiol Behav 21 785 1978).

It took place mainly during the dark hours, with marked increase prior to the beginning of the light hours. The increase was attributable to the consumption of very large meals.

In rats which were partially REMS-deprived, the large meals were shifted toward to the middle of the dark period and an increase was observed in the frequency of small meals during the light hours.

Total REMS-deprivation in adult rats replaced the well-organized meal pattern by frequent small meals distributed evenly throughout the 12/12 h LD cycle. When the REMS deprivation was finished, the usual meal pattern returned.

The results suggest that the correct timing of the consumption of meals in the rat with respect to the LD cycle seems to be dependent upon undisturbed REMS. Total REMS-deprivation completely inhibits the generation mechanism for large meals.

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Hilakivi I and Leppävuori A (Department of Physiology Univ rsity of Helsinki, Finland)
OPPOSITE INFLUENCES OF ALPHA- AND BETA-ADRENOCEPTORS ON PARADOXICAL SLEEP IN THE CAT?

A relatively specific α-blocker phentolamine (PHE), increases paradoxical sleep (PS) in the cat (Putkonen, P and Leppävuori A Acta physiol 1 scand 1977 100 488), whereas nonselective (β_1 β_2) highly lipid soluble β blocker propranolol (PRO) decreases PS (Hilakivi, I et al Med Biol, 1978 56 138). Atenolol (ATE) is a β_1-selective poorly lipid soluble blocker with claimed weak entry into the brain.

The combined action of PHE with PRO and ATE was studied in cat with permanent electrodes for EOG EMG and ECoG. Sixteen hour polygraphic records were taken after intraperitoneal injection of 10 mg/kg of PHE alone or preceded by 5 mg/kg of PRO ATE as the two have the same β-blockade potency ratio.

PRO counteracted the increase of PS by PHE (p<0 01; t-test df 10) while ATE proved ineffective. The 16 hour values for PS (control 15 3%) were PHE 20 7% PRO+PHE 16 4% and ATE &PHE 22 9%.

The failure of ATE to oppose PHE is most probably due to its low access into the CNS. The binding of PRO to 5-HT-receptors demonstrated in rat brain membrane might be sufficient PS-disturbing factor to account of PHE. Here directly PHE and PRO could affect noradrenergic transmission either by modulating noradrenaline (NA) release or by altering the neuronal responses to NA at postsynaptic level in the cerebral cortex, where opposite influences of α- and β-receptors on firing rate have been described (Bevan P et al Br J Pharmac 1977 59 635).

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Spinweber C., Ursin R., and Hilderbrand R L (Naval Health Research Cente San Diego Cali fornia USA and Institute of Physiology University of Bergen Norway) THE EFFECTS OF L TRYPTOPHAN ON WAKING EEG AND DAYTIME SLEEP IN NORMAL HEALTHY SUBJECTS

The amino acid l tryptophan has been shown to significantly reduce nighttime leep latency in normal and insomniac subjects Since nighttime administration may accentuate a natural peak f plasma tryptophan tudy was done on the effects f exogenous l tryptophan on waking EEG daytime leep and plasma amino acid levels

Twenty subjects were tudied on two occations one week apart after placebo medication and after 4 g l-tryptophan Half of the subjects were studied in the morning and half in the afternoon

In the combined group there were ignifi cant change towards lowing of waking EEG (eyes open) frequencies studied 30-40 min after the medication was given before lights out for the nap Self-evaluation f activation and leepiness did not differ between conditi ons Sleep latency (time from lights out to first stage 2) was ignificantly reduced after l-tryptophan (mean 12 6 min placebo mean 23 6 min P < 0 01 Wilcoxon matched pair igned rank test) There were no significant diff rences in the composition of leep between th two conditions The were no difference in EEG leep parameters between the morning and afternoon groups Plasma amino acid levels are being analyzed.

It is concluded that l tryptophan in the employed dose has deactivating ffect on the waking state and does not affect leep per se

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Alihanka,J. (Department f Physiology University f Turku Finland) APPLICATION OF THE STATIC CHARGE SENSITIVE BED (SCSB-) METHOD FOR LONG TERM MONITORING OF AUTONOMIC NERVOUS CONTROL OF HEART RATE,RESPIRATION AND BALLISTOCARDIOGRAM

The SCSB-method for recording body movements during sleep has been described earlier (J Ali hanka and K Vaahtoranta EEG lin Neurophysi l 1979 in pres) When the patient is lying motion-les simultaneous monitoring f heart te res piration and ballistocardiogram (BCG) i possibl by using three different output channel with different time constant The changes in SCSB-BCG was compared with the imultaneous recording f ECG FCG and impedance cardiography in 6 subjects after ergometri workload and the changes in the amplitude and f equency of the SCSB-spirogram were compared with imultaneous spirometri recording The SCSB-BCG we found to exhibit imilar balli ti complexes and similar respi atory variation as has been seen with previously known methods Vari tions in the amplitude reflect the changes in the output of the heart The changes in SCSB-spirogram correspond to the changes in the spirometric recording SCSB-recording was made during 20 nights in 10 normal subjects imultaneously with EEG EOG EMG and ORrecording In different sleep stages typical patterns in the SCSB-recording make quantitative leep tage scoring possibl with good ccuracy on the basis of the SCSB- ecording only SCSB-recording can be done even at home in the own bed f the patient using imple apparatus of an ampli fier and small C-cassette tape recorder (g during 3 nights) Thus the SCSB-method could serve as an inexpensive impl polyg aphi diag nosti examination of many disturbancies in car diac respiratory autonomic nervous and leep mechanisms Grants Juho Vainio Foundation

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Koskiniem A and Ahtee L (Division f Pharmacology Departmen f Pharmacy University f Helsinki Finland) EFFECT OF TAURINE ON THE LOCOMOTOR ACTIVITY OF AMPHETAMINE AND APOMORPHINE IN MICE

Taurine is pos ibl inhibitory neurotransmitter in the CNS (Oj Lähdesmäki Med Biol 1974 52 138) which decre cerebral dopamine (DA) release (Garci de Tabanes Froue al Naunyn-Schmiedeberg Arch Pharmac 1978 304 95

We tudied ffect f taurine on hyperacti vity induced by amphetamine apomorphine Taurine saline was admini tered intracerebroventricul ly (icv) to femal mice Amphetamine (1 0 2 5 and 5 0 mg/kg) and apomorphine (3 mg/kg) were given ip 1 mi bef re the icv injection Apomorphine mice were pretreated ith reserpine and αMT Taurine (1 μmol per mouse) decreased the activity by abou 63 % (P<0 01) during the fir 30 min but not any late time poi t tudied Taurine antagonized the timul tory ctivity f 2 5 mg/kg f amphetamine by about 46 % (P<0 001) during the first 30 min but not l te and that f 5 0 mg/kg f amphetamine during 30 min by 58 % (P< 0 001) 60 mi by 56 % (P<0 01) and 90 mi by 41 % (P<0 05) fter the admini tration of the drugs T urine did not alte the locomotor timulatory activity f apomorphine in reserpine and αMT pretreated mice The dose f taurine used did not al er cerebral DA concentr tion or αMT-induced DA decrease

Taurine could antagonize amphe amine induced locomoto timul tion by decreasing amphetamine induced DA releas

Supported by the Sigrid Jusélius Foundation

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Lew de , T (Dept f Ph rma l gy F lty f Ph rmacy, BMC Uni rsity f Upp l , Swede): AMPHETAMINE INDUCED HYPERACTIVITY COMPLETE AN TAGONISM WITH R 4 1284 A CATECHOLAMINE (CA) STORAGE DEPLETOR

R 4 1284 be z q i li i d i ti hi h pid b l CA d pl tie i hibiti g CA ynth i pt rs t t d f ph t i t gent A d d time d pend t i hibiti f amphet i i duced hyp ti ity, me ed ith Ani ti ity met ent i ed Compl t t se lim f 4 mg/kg f d amph t i 50 bs d t l h ft 40 mg/kg s c f R 4 1284 Under th condition b i CA er d pl t d t <90% f t l l l Th e en li rel ti hip b t ee th d gree f R 4 1284 i duced CA d pl ti d th mph tami tage l Admi i t tion f T 40 mg/kg f R 4 1284 t l h ft amph t i compl t ly b li hed th going hyper cti ity ith i 6 12 i tes

Th p t t lt ill b disc d d omp red ith th ompl t mph tami t gonp ti l t geni ti action f ti thyl p-tyro ine d the des whi h 50% d>90% depl ti re pe ti ly f b l CA

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Oj , S S and Kontro, P (Depa tme t f Bi medical Sci U iv rsity f Tamper F n land) EFFLUX OF TAURINE FROM RAT BRAIN SYNAPTOSOMES

Taurin has bee con de ed neu otrans mitte ne omodulat in th CNS A d pol ization f p esynaptic memb en libe t taurine f om ti ues but this relea has not bee hown unequivoc lly t be cal cium-dep ndent lik neurotransmitt e- l e h ld be W ha now d ff renti t d th ynapto omal fflux ompone t f t uri in rde t tudy th i 1 di idual p ope ties Synaptosomes l l t d from t bole b i we fi t p l ded f 60 mi ith [3 S] t i (50 µmol/l 1 mCi/l) t 310 K und r O in K eb Ri g HEPES gluco medium (pH 7 4) and then upe fused as synapt some bed nd wi hod betwe two myl ne het Th pontan us fflux f [3 S]ta in w low nd c uld b d s ibed s omp i ing two fi t-orde rat mponent f hi h th lower on may represent the rel as f i t syn pt omal ta ne Only ry high ta ri con entration i the upe fusat wa bl t h nc th fflux Depol i ing pota ium i oncentration timul ted it and induced th ppearanc f new int rmed t fflux component Th ompo ent ab t hen cal ium i ns were omitted f om th medium The ompon t may th rigin t f om the empty g f ynapti ve les It d es t h es ily itne fun ti f t ine as neurotrans mitt in unaa rted ynapti i les tai much tauri whi h co ld be l b rat d t geth with prop urotransmitt upon p synapti depol rizat

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Kaakkol S nd Kääriäinen I (Department f Pharma l gy Unive ity f H l inki Fi land) CONTRALATERAL CIRCLING BEHAVIOUR INDUCED BY INTRANIGRAL INJECTION OF TAURINE.

Ta rine ha be n proposed t b an inhibit ry rotransmitt -modul t Th brain ontains l rge amounts f taurin pe ially tri tum nd ub tanti nigra W tudied the beh ioural ffect f unilat ral intranigral i j tion f t urine in t T urin (1 2 µl) inj ted through an- nul i t th ub tanti nigra f conscious rat T uri (10 200 µg) ed d e-depen- d tral t ral iroling f bout 4-5 h. Pretreatment ith bi uoullin (3 mg/kg i p) tryohni (0 25 mg/kg i p) inhibited ignifi tly ta ine ind ed iroling Atro- pine-pretreatme had no ffeet ireling O 5 mg/kg f hal perid l had no ffect but 1 mg/kg partly inhibit d taurin induced ircling Th unil teral intranigral injecti f t rin ea sed tral t ral iroling lik the inhibit ry rotransmitt GABA (Scheel Krüg et l N uro i Lett 4, 351 356 1977) S ry hnin nd bi uo llin antago i ed th t ri induced iroling they inhibit the hypoth rai (Sgaragli G P In T ine and Neurologi l Di rd rs 1978) and l tro- phy i l gi l ff t f taurine i brain t (Curti et al Brain R 33 57 73 1971) T urine may have ome infl ene on the fun tion f th xtrapyramidal sy tem.

(Supported by Research Foundation f Ori yhtymä)

Kirs A L nd Karppanen, H (Departme ts f Pharma l gy Uni ity f Oul and Uni rs ty f Hel ink Finl nd) CENTRAL CARDIOVASCULAR AND THERMAL EFFECTS OF ARACHIDONIC ACID IN RATS

Arachidon d (AA) i th precurs f prostagl di $F_{2\alpha}$ ($PGF_{2\alpha}$) and th omboxa A_2 (TxA_2) i the b ain ti sue (Abd l Halim,M S t l Pro t gl ndi 14 633 1977) Ce t lly dmin t red $PGF_{2\alpha}$ i duced dos -depend t ri of blood pre sure h rt rat nd body tempera tu (Karppe H t al Pr t gland ns 1978 in pre) I th work th ff t f t l ly admi i t d AA blood pre su h rt ra t and body tempe t re re t d d ur the se-an th t d t W I tud ed th i fluenc t dometh nd p rac tamol hi h known inhib t rs f p t gl ndi synth i th brai th tral ffects f AA Sinc imids l npe f inhib t f TxA_2 ynth (M edlema P t al P oc Natl Ac d S i 74 1716 1977) t ra t th th at I ffect f AA I t d i d Th admini t t on f 0 01 100 µg f AA in t th ght l t ral ri l f the brai ( ) du ed dos l ted incre i blood p re nd body empera w th i abl ff t on h rt Pr tme t th ndometha in para tamol (100 µg/ t) bol hed th thermog ff f t lly dmi te d AA I dometha d d t ffect th cardiov cul ha g nduced by AA I d l pret tme t (100 µg/rat) pa tly t gon d th entral hypert as ffect f AA but f iled t lt the th rmal ff t f AA Th results sugg t that the tral th rmo geni ffect f ch don acid may b du t it on rsion t prost gl ndin Th at l hype t nsive ffect f ach do d may b t l t partly be du t the f rm t on f TxA

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K I Rapakko S and Al k l M M (Dept. f Pharma and Phar , Univ Kuopi , i land) EFFECTS OF SOME β-CARBOLINES ON PHENETHYLAMINE AND APOMORPHINE STEREOTYPIES IN RAT

Ac rd g t th DA hypoth f paranoid hi ph eni the t eotyp b ha r (SB) in- duc d by d pami gi drug g amph tam ne and pomorph (APO) as well β-phenyl thylam (PEA) ha been ed nimal mod l f schizo- phrenia Some t t hyd β-ca boli es l ted to h llucinoge i H rmal alcal id y b f rmed i th huma body Th mai pharmacol g l ffect eem t b d t indirect rotonergi ti W h ve mpared th ffect f ome β-ca b li SB ed by PEA (50 mg/kg p) nd APO (2 g/kg i p) n l W ta rat β c boli (25 g/kg p) we gi en t gether th APO PEA i th p li rv t t The do ffect el ti hip wa tudied from he mo t pot t β-c boli Ev luati f APO-i du d SB b ed h numbe f b t /30 sec 20 30 d 40 i ft th dmini t o f th drug SB ft PEA wa l ted ith peci l l (Bori t l 1977) All th β ca bolin studied inhib ted APO t eotypy 6-Meth yharmal wa th mo t ff ti f l low d b β bol (BC) t t hyd β-carboli (THBC) 1-meth l THBC 6-m thoxy THBC and 6 hy- droxy THBC 6-Methoxyharmal BC and 6-hydroxy- THBC ff ti i PEA t eotypy 6-Meth- xyharmalan wa mo pot t than BC but th ma mal ffect we the ame THBC 1-methyl THBC nd 6-metho THBC d d not how ignifi cant ff t Th PEA nd APO t eotypi re not dent l nd β boline did no inhib t them th ame way Thus th mod f PEA and APO tereotyp em t diff If th DA hypoth i lid th β-ca bol y pre g t ra her tha wo en p amoid psycho es

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J DR. P — Josefsson J -O. (Department of Pharmacology University of Lund, Sweden) RAPID DEVELOPMENT OF TOLERANCE TO OPIATES IN AMOEBA PROTEUS

Previous studies show the nanomolar concentrations of β-endorphin met-enkephalin and morphine reduce the intensity of pinocytosi induced by Na^+ or tris$^+$ In addition to being inhib ted by the narcotic antagonist naloxone the opi te effect i reduced by caffeine and Ca^{++} and potentiated by calcium antagonists (verapamil 600 NTH)

In media containing β-endorphin or morphine the amoebae develop tolerance the opiate effect the degree of which i rela ed the duration of treatment (few hours) and the dose of the opiat (40-400 nM morphine) Cross tolerance develops for β-endorphin, met-enkephalin and morphine Tolerance does not develop in tarved cells or in cell trea ed with cycloheximide an inhibitor of protein synthesis.

After induction of tolerance the cells are dependent on the presence of opiates or normal pinocytoti activi ty and withdrawal of the opi te or addition of naloxone is followed by period of blockade of pinocytosi Normal pinocytotic activity i restored by opiates EDTA or verapamil whil Ca^{++} caffeine or dbcAMP potentiate the withdrawal effect

Pentazocine (400nM for 15 hours) has acut effect on tri$^+$ induced pinocytosi similar to morphine but induces lower degree of tolerance which does not lead to signi ficant dependence Like naloxone and nalorphine pentazocine precipitates he withdrawal effect in morphine tolerant cell

I is concluded that amoeba opiate recep ors discrimi nate between different narcotic analgesics, tolerance and dependence developing onl to the agonisti opi tes It i suggested that acute blockade of pinocytosi by opiates is caused by reduction of the cellular activity of Ca^{++} or decreased sensitivity to this ion. The opposite situation which leads to Ca^{++}-blocked pinocytosis prevail during the withdrawal period

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A il L M J nd Ah , L (Di i ion f Ph rma l gy Dep nt f Ph rmacy U i i y f H l inki Fi land) EFFECTS OF CHRONIC MORPHINE ADMINISTRATION ON CA ECHOLAMINES IN DIFFERENT PARTS OF THE RAT BRAIN

Mal Wi we h i lly ed wi h ll (on rol) morphi (10-20 mg/kg wi d ily) f 20 d y f llowed by wi hd l pe iod f om 1/2 6 day Dopami (DA) nd nd enalin (NA) ncen i we sured i fi b in p li bi p (LIM) ry i ta (STR) h i pho di pha- lon and l we b i em (LBS) The f DA nd N d pl ion wa sed i g -m hyl p- yr i (aMT 200 mg/kg i p)

N i he DA no NA ne ion ha g d by wi hd l f morphine lin The h n- i morphi (wi hd wa f 1-4 d y) aMT ind d DA depl i lowe han h f he l i LIM b mo i STR Mo phi d se (10 mg/kg 2 1/2 h) as l ed he aMT ind d DA d pl ion i LIM d STR f h i morphi nd nali 6 8 l ion f MT i d d DA depl ion by he h ll ng d i 86 ed i hr f mo phi he in he on l i LIM he STR aMT d d pl i f b l NA bo q lly i morphi nd on ol ra NA d pl ion rd d by he dose f morphi i ll fi b i i l 2 d y f morphi i hd l i en i h l ed d ple- i i LBS ly i h l

Th ha g h rved i b l A d pl i igh b ib hi i duced by morphin i hd wal l b ra on by we d y weigh l

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Bergslien H Bole E and Rei helt K L. (Inst f Phy iol Uni of Bergen and Inst f Paedi tri Research Rikshospitalet Oslo Norway) URINARY FACTOR IN SCHIZOPHRENIC PATIENTS STIMULATES OPIATE RECEPTORS AND DOPAMINERGIC SYSTEMS

Urin from hizophren pat ent wa precip ated with benz i acid and fractionated on Sephade G 25 and P 2 g ls and char t ri ti pa terns f peptide and prot in associ ted peptide complexe were found One f ot that showed trong bi logi i ity (factor Ib2) wa further tudied When injected int erebrov ntricula ly, f or Ib2 induced characterist behavior l yndrome including explosive motor beha ior", au onomi changes, and longlasting (at leas 1 week) cat l psy igidity and loss f igh ing refl xe P i en i ty wa strongl reduced Ra wi h un l t ral 6 hydroxy-dopamine l on f the nigrostr l pathwa howed urni g ipsilateral t the le on ide f er fac or Ib2 Most f the behav oral ffects were partly or compl ly blocked by nal one and/or h loperidol Re peated ject on f f or Ib2 resulted in t l anc dev l pment as also cros l rance with morphine I wa concluded hat ach o- phreni pa ore in he pe pep id peptid l k fac or ha ha strong opi recep or mul ing ff and lso reral in dopamin rg mul on Th pos ibility hat hi f or i f importance in he pa o- genes in ach phreni wa d cussed

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Kanto J Koelu, M and Lamminterepta R (Department f Pharmacology University f Turku Turku, Finland) STIMULATION OF HUMAN GROWTH HORMONE (GH) SECRETION BY DIHYDROERGOT AMINE (DHE)

Ergot alkal ids are group f drugs whose main peripher l pharmacological action consi t f smooth muscl timulation and interference with lpha-adrenoceptors Many f them re reported to have endocrinological ffects DHE i hydrogenated derivative f ergotamine which mainly acts an alpha-blocke but in some rgans exerts lso an lpha timulant otion and is relative trong serotonin antagoni t It is sed linically in treatment f rthostati hypotonia and migraine Little i known f its endocrinological ffects in man We now report the GH secre ion timulating influence f DHE

Six volunteers (2 females 4 males) were tes ted After an overnight f st they were given intravenously 1mg DHE (VASOCTIN Leiras) 0 9% saline injections Blood was collected by venepuncture before and 15 30 60 90 120 and 180 min aft injections Serum GH was analyzed by RIA. It wa found that DHE ignificantly timulat GH secretion serum GH level rose from the mean basal value f 3 7±0 75 (mean±SEM) ng/ml to the maximum mean value f 19 4±6 52 ng/ml t 60 min (p<0 05) Afte saline no ignifican GH ise was observed

The mechanisms f action f DHE on GH secretion emains to be larified According to ani mal tudies DHE has only minimal ffects on dopaminergic transmi ion The DHE hardly timu l tes GH release is dopamine Possibly DHE stimulating ffect on central al which ha been earlier hown to mediate GH l se

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K mg L , Mi i A L , Lammi t ast R L ppl A d Nieminm L (F rm s Group Ltd Res rch C t L ake-Medipol a d De- partme t of Pharma logy Univ rsity of Turk Fi l d): DISTRIBUTION OF METOPROLOL BETWEEN PLASMA AND SALIVA

Metoprolol (M) bet blocki g ge t s determi ed i pl a d sali of 10 h lthy ol teers fo 0 24 h s well as in pl sma s liva d s li ary gla d of 3 at 3 h after a i gl admi istrati n p o Th huma dose s 100 mg d the ani l dos 50 mg/kg

The co ent ti of M were det rm ed by GLC usi g it g electi n det to

Th sali ry l l f M ere equel ly and sig ific tly highe th th plasm le els F th pe k le l in l t s were 646 156 d 121 32 ng/ml (me n ±) Th mea ratio sali a/pl sma of all s mpl s w s 5 l Th lf tio h lf life as si il in both fl id (me s 2 6 nd 2 0 h, resp ctively)

Th l els of M in at pl ma ere 93 648 (me 384) g/ l Highe n trati ere found i aliva d i saliva y gla ds ra gi g 260 853 d 250 837 ng/ l r pe ti ely Th se fi di gs h th t l) M i cumul ti g te i ly i t th li ry glands d 2) dist i buti f M betwe plas d ali a t k t pla by imple pa i diff i b t thro gh bi ding t th liv y gl d

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Sage , G, Jacobsen S nd Hansteen, V (In titute of Pharmacology Unive ity f Oslo and Medi l Department VIII Ulleval Hospital Osl Norway) ANTICOAGULATION AND PLASMA PROTEIN BINDING OF PROPRANOLOL

Propranolol is extensively bound in plasma 1 acid glycoprotei (1 AGP) i the mai binding protein but propranolol i also distributed to lipoproteins [1] These two protein f ctions ar respon ibl f 60-75% and 5-25% of total binding respectively Binding f propranol l wa tudied in plasma from 35 pati ts recei ing anticoagulation the apy (war farin or heparin) after myocardial inf rction Plasma binding wa d termined t propranolol concentration of 125 ng/ml by equilibrium dia lysi against Krebs Ringe buffer pH 7 40 37°C Free f tty acids (FFA) were determined by col imetric method [2] The unbound f ctions in plasma were 0 065 0 013 and 0 078 0 015 (2 5%) and the FFA level were 0 6 0 4 and 1 9±0 7 meqv/l (0 5%) after war farin and heparin administration spectively Increase in FFA i xpl ined by heparin acti vation f lipoprotei lipase (LPL) Positive linear correlation was found between unbound fr tion and concentration f FFA (2 5%) In vitro addition f heparin or FFA [3] did not affect the binding Concentrations f 1 AGP were not ignificantly diff rent i the two groups (5%) The present esults indicate that the ctivation f LPL decreases lipoprotein binding of p opranolol

Ref rence 1 Sager G Nilsen O G and Jacobse S Biochem. Pharmacol (in press) 2 Lauwerys R R Analyt Biochem 32 331 (1969) 3 Spector A A and Hoak J C Ana lyt Biochem 32 297 (1969)

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And es en F Bjerregaard P Kornerup H J Lederball Ped rsen O Mi kelse E, Pedersen E B nd Week J (University f Aarhus Denma k) BIOCHEMICAL CHANGES DURING PROPRANOLOL TREATMENT

Thirty patients (women and 14 men) with WHO hyper tens on were randomly alloca ed to one f three groups treated during 8 weeks with propranolol In preceding run-in period the dose was gradually increased to 40 mg 4 times dail (group 1) 80 mg 4 times daily (group 2) or 160 mg times daily (group 3) The ave age lasma concentrations of propranolol (af er the 4 week adjusting period after weeks and afte 12 weeks) were 66 75 78 76 and 54 ± 64 ng/ml (± in group 1 71 137 183 15 and 162 123 in group 2 and 296 137 390 11 and 340 37 ng/ml in group 3 The protein concentration in serum decreased in 9 f the 10 patients in group 1 No consistent changes were observed in the two other groups On the average the concentr ti on f creatinine in serum increased with 0% The crea tinine learance decreased in group but d no change ignif cantly in groups and The concentration f and um in serum and ts excretion in the urine di not show any consistent changes wheres the potassium concentr tion serum ose the two groups given the lower doses but remained unchanged in the high dose group (all women 75 ► 00 all men 08 ► 30 ml/ l) Serum cholesterol showed no change tr lycerids e vels increased in groups 2 and 3 Serum uric acid concentration showed an average incr ase in al three groups from 5 0 to 5 6 mg/100 l The percentual increase was highest in group 1 and lowe group 3 γ-con centration in plasma ll in al three groups with an average f 20%

Acknowledgements Supported by the Danish Hear Association.

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S nd R R So g al and l sen B, (Department ts f harm cology nd Ph rmace ti l Chemi try I i te f Ph rmacy nive i y f Osl Norw y) ILIARY AND URINARY EXCRET ON OF S CO YL DERIVATIVES IN THE RA

llowing p tudi ryphe i d i ti (nd l he ph rma ol eniol 1979 258) ylb oodyl (I f di phenol) and its di t ith wa i id (i osdyl) and lphuri id (III-pl sulph) we give by toma h tube f ed d 3 l μmol pe 100 g il cell ed i th pe iod 0 h 6 12 h and 12 18 h f llow ing dmi i ti nd y d to al (f p jug ted) by LC thed ith l pe i l and d Me f i ns (% f d t) ed i 5 pe sen- po nd nd period we I 41 2 ± 4 3 6 8 ± 4 7 and 25 l 2 5 74 0 ± 4 7 51 9 ± 7 9 nd 30 8 ± 2 5 I 9 0 ± 0 9 28 0 ± 5 4 nd 19 6 3 l aly ino amounts we p f Sampl k by bladd pe ur ed y ed above thermo hoved th th iary er f l i ignifies compared amo or ed i bil No I wa found ine 0-6 h af ad i i ion f I 7 μmol pe 100 g i fed ed di he me p ly ha ithe nd I The re lts d mo tr tha I nd I ff ive ly ab bed d i g th i nall i tinal tras i nd ed in the bil Th be rption f howeve tak l ce mo di tally i l ompl te nd i p obably due t enzymati hyd ly i f thi p odrug ti I i er dan th the ssumpti f th l (Ar h Ph rma l 1972 2 4 6)

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Laomp, P.V, Palkonen, R O and Sotaniemi, E.A (Clinical Research Unit Department f Medi ine and Department f Pharmacology University of Oulu Finland) PLASMA HIGH-DENSITY LIPOPROTEIN CHOLESTEROL AND HEPATIC DRUG METABOLIZING ENZYME ACTIVITY IN MAN

Recent tudies ha linked high-density lipoprotein (HDL) cholesterol with the development of atherosclerotic diseases Plasma HDL cholesterol level i in inverse elationship to the incidence f coronary heart disease Some compounds such alcohol and phenytoin increase the plasma HDL cholesterol level The effect might be related to the stimul tory ffects of these compounds on the activity of hepatic microsomal system To test the hypothesis we compared plasma HDL cholesterol level with hepatic cytochrome P-450 content and with the rate f antipyrine elimination from plasma in patients with diagnosti liver biopsy

The results showed positive correl tion between plasma HDL cholesterol level and cytochrome P-450 content in the liver and also between HDL cholesterol and the antipyrine limination rate Pl sma HDL cholesterol and hepati cytochrome P-450 concentrations we related to liver histology

The esults suggest that there is rel tionship between the hepatic production and the plasma level f HDL cholesterol The findings also suggest that hepatic cytochrome P-450 reflects liver apacity for both HDL cholesterol production and drug hydroxylation

Grant Finnish National Research Council for Medical Science (Academy f Finland)

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Pauppanen R , Lammintausta, R. Viinamäki O Anttila, Laine R. and Viikari J (Departments of Pharmacology and I ternal Medicine University of Turku and Research Center Lääke-Medipolar Turku) STEADY STATE PLASMA LEVELS OF CHLORPROPAMIDE AND ANTIDIURETIC HORMONE IN DIABETIC PATIENTS

Chlorpropamide (CP) i used in the the py f diabetes insipidus and it is also known to induce an inappropriate ADH-secretion syndrome in some patients receiving it as an antidiabet gent The purpose of this tudy was t investig te whether there is correlation between teady state levels of CP and arginine vasopressin (AVP) in patients (n=25) having diabetes mellitus CP was given 125-750 mg/day CP was measured spectrophotometrically and in some cases also gaschromatographically (GLC) As previously known the interindividual variation f the CP plasma concentration after equal doses was large The plasma levels anged from 31 t 211 µg/ml (118±54 µg/ml) The specif ic GLC procedure gave in low concentrations imilar results but in highe concentrations the GLC tended to give lower values The value of AVP ranged from 2 8 to 21.3 pg/ml (x 10 6± 5 5 pg/ml) The mean AVP value f these patients did not diff ignificantly from the mean fasting value of the controls (9 7±4 5 pg/ml n=25) No correlation between plasma concentrations of CP and AVP was found (r=0 22) Others have shown the CP has an antidiuretic action lso in some normal persons and diabeti patients CP has been suggested to act by increasing the synthesis and release f ADH In this material f diabeti patients no ignifi cant change in the plasma ADH level was found during the chronic administra ion of CP

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Salonen, J.S (Department f Pharmacology Institute f Biomedicine University f Turku Finland) PLASMA LEVELS OF PROCAINAMIDE AND N-ACETYLPROCAINAMIDE DURING MAINTENANCE THERAPY

The steady-state levels f procainamide (PA) and its active metaboli N-acetylprocainamide (NAPA) in plasma were tudied during maintenance therapy with sustained release PA Both concentrations were imultaneously determined using gas-chromatography with nitrogen sensitive detection Sampl from 40 hospitalized patients receiving an average daily dose f 2500 mg PA (range 800-3600 mg) were analyzed The patients ages varied between 42 and 81 years (mean 61 years) and majority of them was suffering from myocardial infarction

The steady-state PA level t the time f dosage ranged from 0 5 to 10 5 mg/l and 2 h fter dosage from 1 4 to 16 8 mg/l Below the proposed therapeuti range 4 10 mg/l (Koch-Weser, Clin Pharmacokin 2 389 1977) were 75 and 54 % respectively Concentr tions les ly exceeding 10 mg/l were found during the absorption phase only The NAPA level ranged from 0 5 to 10 6 and from 0 8 to 13 7 mg/l respectively The acetylation ratio C(NAPA)/C(PA) ranged from 0 4 to 3 7 and was les than 2 in 77 f samples the time f PA dosage

Many f the patients were receiving reduced PA dosage because f renal dysfunction heart insufficiency Howeve there seems to be light tendency towards subtherapeutic plasma concentrations f PA, which would indica the need fo more regul pl sma l vel de rmination Conclusions about the contribution f NAPA to the therapeuti fficacy adverse reactions in these pa ients ill have to wait for furthe investigation

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Kerppanen M I Myllylä V V and Laomp P.V (Departments f Neurology and Medi ine Uni rity of Oul , Oulu, Finland) INTERACTION OF SODIUM VALPROATE AND CARBAMAZEPINE AS REFLECTED IN SERUM DRUG LEVELS.

Sodium valproat (VPA) i new id ly ac pted as an effecti drug in petit mal epil psy and also in grand mal epil psy especially in combination with the anti convulsants. The major pathway of metaboli transformation of VPA i conjugation Th chroni treatment with anticonvulsant often results in steady state level of drugs in th body Th present tudy was und rtaken to evaluat the antiepilepti therapy ith the combination f VPA and carbamaz pin (CA) as refl cted in serum drug levels.

Serum VPA level was d termined by enzyme immunoassay and that of CA by gas-chromatographi method Therapy with VPA alone resulted high VPA level than tho measured in patients wh were treated with VPA in combination ith CA Serum VPA lev l in patients treated with the combination were in most case t suboptimal lev l Serum CA lev l in patients treated with th combination f VPA and CA or with CA alone did t di erg from each ther

The result uggest that CA enhanc the clearanc f VPA from the body In thi connection low VPA lev l may reflect the CA-caused timal tion f hepati metabolism f VPA

Grant Finnish National R earch Council for Medical Science (Academy f Fir

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Li dhem L.O F sb rg T Mell t Bm,W , Mal t sy,J , Og e ,S O d Spe be B (Dep f Ph rm ol R e ch d De el pm t Laborato ies AB A tr Läkemedel Söde tälje Swede): CARDIOVASCULAR EFFECTS OF ZIMELIDINE AND TRICYCLIC ANTIDEPRESSANTS IN ANESTHETIZED AND UNANESTHETIZED DOGS

Zimelidi (ZIM) is a ne antidep e sant ith a s le ti e ff ct on th ne e l ptake f 5 HT Ca di s l effect of ZIM a d amit ipty-li e (AMI) lomip ine (CLOMI) and i ip amin (IMI) aft i i f io in dog we e studied In esthetized mo g el d gs 0 5 mg/kg/ i of AMI CLOMI d IMI induced de ea s in mean rt i l blood pre su e (MAP) l ft nt ic lar d ystolic p ess e (LVSP) rtic flow (AF) card work (CW) and co t actility d strok ol e (SV) whil left t icul endd i t lic pre su a d ight t i l me press e incre s d AMI and IMI i cre ed nd CLOMI de rea ed h a t ate (HR) ZIM d c eas d MAP d periphe al sist ce and inc s d pulse press re t 0 5 mg/kg/ in At l 0 g/kg/ in als LVSP AF, CW HR nd SV we red d The lethal d se of ZIM I 5 ti es th f th tricycli s in o t st t ZIM the t icy li s induced ma ked ECG h g s I a sth tized beagle AMI a d ZIM we i f s d at 0 5 g/kg/ i ZIM induced a lo ly de loping d ea e i pe iphe al re sta ce d a inc s i AF vi a increas i HR AMI inc sed HR kedly and red ed SV while pe ipher l i ta fi st i c e ed then dec d MAP as t a i ntly i r ased

It s l d d that the t icyclics induced effects the h a t leadi g to o gesti e he rt f lu ZIM had small h t effects but decreased asomot t le ding to shock at ry high d

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Mikk lsen E Lederballe Pedersen O. Andersson K -E Kjlsbek Pedersen E Østergaard, R. (Department of linical Pharmacology University of Aarhus and Department of Surgery L, Aarhus Kommunehospital Aarhus Denmark) IN VITRO EFFECTS OF PRAZOSIN NIFEDIPINE AND DIHYDRALAZINE ON ISOLATED HUMAN MESENTERIC AND PERIPHERAL VESSELS

The inhibitory and relaxing ffects of prazosin, nifedipine and dihydralazine on contractions induced by noradrenaline (NA 1 8 10^{-5}M) or potassium (K^+ 127 mM) were investigated in isolated human crural and mesenteri rteries and veins

Vascular ring prepar tions were suspended in organ baths and isometric tension was recorded.

Prazosin was the most potent of the drugs in counteracting NA induced contractions in all types of vessels except peripheral veins where the effect of nifedipine was the more pronounced Prazosin was completely devoid of relaxing or inhibitory effects on K^+ induced contractions Nifedipine was by far the most effective f the drugs investigated in counteracting K^+ contractures in all types of vessels

Nifedipine had more marked effect in venous than in arterial preparations from the peripheral circulation whereas no such difference was seen in mesenteric vessels

Dihydralazine was found to have very low potency in all types of vessels and no difference was observed between arterial and venous prepar tions

The results suggest tha the in vitro effects of the three vasodilatators are markedly different from the in vivo actions Thus the correlation between the in vitro effects on arteries and veins is quite different from tha reported in clinical studies on prazosin, nifedipine and dihydralazine The linical usefulness of dihydralazine cannot be explained from the poor in vitro effect of the unmetabolized drug

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Edvi sson L Brandt L Andersson K E nd Bengtsson, B (Department f Clini l Pharmacology and Neurosurgery University of Lund Lund, Sweden) INHIBITION BY NIFEDIPINE OF CONTRACTILE ACTIVITY OF HUMAN CEREBRAL ARTERIOLES IN VITRO

Recent studies have demonstrated that cerebrovascular smooth muscl both from man and animal espond t asoconstricto and asodil tor gent In ituations whe an intense constriction occur asospasm ft ubarachnoid hemorrhage it i importan t find way to lieve thi Ce ebr l art ioles obtained during neuro urgical operations we tudied in itro Ve sel segments 200–500 u in di met wer mounted between two L- haped metal prongs f isometr ecording f cont actil acti ity Dose-r l ted vasoconst ictions wer obtained by noradrenaline 5 hydroxytryptamine exce K tologous blood and plasma Nifedipine (0 1 ng/ml) l ium influx inhibit ffectively dilated th rteri les const icted bef rehand by eithe of the above mentioned asoconstricto agents The dose-response curves to no adren line and 5-hydroxytrypt min were fte ifedipine treatment duced t about 25 per cent of control

On the bases of these expe iments it ug gested that the contractil action f no adrenaline 5 hydroxytryptamine K ut logous blood and plasma involves an influx f calcium from the extracell lar medium and that block ade f thi influx by ifedipine leads t re laxation f the ve sels It might the fore be peculated whethe se f lcium antagonist can be value in the under tanding f the pathogenesi of cereb al ascular spasm

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Norn S , Sko P S , Gri le A, Klysne , R. and Karups K.W (Department f Pharmacology University f Copenhagen, Denmark) PLATELET SEROTONIN RELEASE REGULATED BY H_1 AND H_2 RECEPTOR STIMULATION

Recent research has shown th t different leukocytes are provided with hi taminergic H_2 receptors and possibly lso with H_1 receptors and that stimulation of these receptors can regul te the leukocyte function and thereby the all rgi i flammatory response

In the present study we have i vestigated whether the platelet function can be regul ted vi H_1 and H_2-receptor stimulation Human platelets from normal i dividuals were resuspended i a Tris-phosph te-carbonate buffer (pH 7 4 at 37°C) contai i g EDTA 6 6 mM and 30 mM of MgSO$_4$ and theophylline KCl 2 5 mM NaCl 100 mM and ... glucose 5 mM 3H-serotoni was i corporated i the platelets and the thrombin-i duced secretion f platelet 3H-serotonin as examined after incubation of the pl tel t suspension with /20 U/ l thrombi t 37°C for 30 sec The release of serotoni as determined as residual 3H-serotonin i the platelet pellets The influence of H_2-stimul tion on thrombin-i duced serotonin release was examined i the presence of equimol concentrations (10^{-4} to 10^{-5} M) of histamine and the H_1-blocki g drug diphenhydramine Both drugs were added to the platelet suspension togethe with thrombi The H_2-stimulation caused an inhibition of the serotoni release which was maximal i the range of 10^{-5} to 10^{-4} M with an inhibition of 30-40% The inhibitory ffect of H_2-stimulation was abolished by the H_2 recepto blocki cimetidine

Simil ly the H_1-stimul tion was i vestigated with histamine plus cimetidine which caus d an inhibition of serotoni release i the range of 10^{-4} to 10^{-5} M The inhibitory ffect of H_1-stimulation was abolished by diphenhydramine These res lts suggest an i volvement of both H_1 and H_2 receptor stimulation i the regulation of the platel t function

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Flyme , R. Gaisle A. Hansen K.W. Stahl Skov, P and Norn, S. (Department f Pharmacology, University of Copenhagen Copenhagen, Denmark): HISTAMINE H_2 RECEPTOR-MEDIATED CYCLIC AMP FORMATION IN HUMAN PLATELETS

Histamine-sensitive adenyl te cyclase has been described i various tissues Thus hi tamine increases cycli AMP formation i gastric mucos uterus heart fat cells and leukocytes by i teraction with H_2 receptor-linked adenylate cyclase Thi ffect i blocked by H_2 antagonists e g. cimetidine In brai it appears that both H_1 and H_2 receptors are associ ted with the formation of cyclic AMP T ur knowl dge the ffect of hi stamine on cyclic AMP formation i human platel ts has not yet been studied. We h ve observed that hi tamine increases the cyclic AMP l vel i human latelets i dose-related manner Maximal stimul tion was obtained with 10 µM f histamine usually giving two-fold increase above basal values Half maximal activation was achi ved with about 1 µM of histamine An increase i the cycli AMP accumulation was obtained with concentration as low as 0 01 µM of hi tamine Thi histamine-induced cycli AMP formation could be blocked by the H_2 recepto antagoni t cimetidine whereas the H_1 receptor blocki g agents diphenhydramine and mepyramine were unable to antagonize the hi tamine response Neithe gent had any ffect on the unstimulated cycli AMP level The hi tamine-i duced cycli AMP response was unaffected by propranol l as well as by the lfa-blocke phentolamine

These res lts suggest that hi tamine can i duce the formation of cycli AMP i platelets by stimulation of H_2 receptors Since the platelet preparation was contaminated with only few l ukocytes (<0 1%) which re however known to posses H_2 receptors the i vestigations will be conti ued with more purifi d platelet suspension

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Tuomisto L and Tuomi to, J (Dept of Pharmacology Uni of Hel inki nd Dept of Pharmacology and Toxicology, Uni of Kuopi Fi l nd) MONOAMINE UPTAKE AND RELEASE IN RAT BRAIN SYNAPTOSOMES EFFECTS OF HISTAMINE AND HISTAMINE ANTAGONISTS

Hi tamine releases adrenaline from the adrenal medul l and both h tamine and its ntagonists have been l found to release monoamines from CNS neurons Hence it wa studied whether or not th effect was direct tyramine-like release t the level f the nerve ending Rat brai synaptosomes (P_2 fraction) were incubated i Krebs-Hensel t b carbonate buffer with 0 1µM triti ted monoamines hypothalamı synaptosomes with 5-HT tri tal synaptosomes with dopamine and cortical synaptosomes with noradrenaline For studying uptake inhibition various concentrations of hi tam ne cimetidine burimamide metiam de mepyramine imidazol acetic cid or imipramine (reference) were included i the incubation medium For studying release the synaptosomes were preincubated for 5 mi with the substrates sedi mented by centrifugation and resuspended and used for second incubation with the compounds studied In two mi uptake experiments only mepyramine was found to be potent inh bitor IC_{50} wa 1 5 µM (DA) to 30 µM (NA and 5-HT) Burimamide inhibited monoamine uptake t 0 15 mM and others were even les potent, imidazol acetic acid was completely inert. I release experi ments hi tamine induced some release both i 2 and 5 mi incubations but h gh concentrations were needed (1 3 orders h gher than those f tyramine) Both imetid ne and metiamide were inert even t high concentrations (1 mM) Hence the results do not give support to the possibility that histamine or the H_2-antagoni ts buri mamide metiamide or cimetidine influence monoamine uptake or release t reasonabl concentrations by tyramine-l ke mechan sm t the nerve ending

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Dahl S G, Hjort M and Hough E (Institute f Medical Biology and Instit f Mathemati cal and Physical Sciences University f Tromsø Norway) THE THREE DIMENSIONAL MOLECULAR STRUCTURE OF ACTIVE AND INACTIVE PHENOTHIAZINE METABOLITES

It has been demonstrated that psychiatric patients on or l treatment with levomepromazine have higher plasma level f the sulphoxide metabolit than f th paren drug (Dahl S G Clin Pharmacol Ther 1976 19 435) Levomepromazine ulphoxide had cardiodepressive effects imol ted rat atrial muscl which we comparabl to the ffects f the paren drug whil the ulphoxide f chl rpromazine was irtually inactive in this as in other es systems (Dahl S G and Refsum H Europ J Pharmacol 1976 37 241)

This study was carried out in rder to reveal any characteristi features i the molecula structure which could di tinguish active from inactive phenothiazine metaboli es The three-dimensional molecular tructures f levomepromazine sulphoxide and f chlorpromazine sulphoxide were examined by ingle-cry tal X ray dif fraction

The molecula tructure f levomepromazine ulphoxide wa imila the previously published structure f chl rpromaz ine (McDowell J J H Acta Cry 1969 B25 2175) In chl rpromazine sulphoxide howeve the ide chai has an entirely different conf rmation in th solid tate This migh expl in th low pharmacol ogical acti ity f chl rproma ine ulphoxide compared ith levomeproma ine ulphoxide and th ir paren drug

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Fo she g T nd Lindbom L O (B p f Ph rma l. R b d B l ment L bo t i s AB A t a Läkem d l Söd tälje, Sweden) SECOND DEGREE AV-BLOCKS AFTER AMITRIPTYLINE AND METHSCOPOLAMINE IN UNANAESTHETIZED BEAGLE DOGS

Amit iptyline (AMI) h be how to i d 2 d d g AV bl k (1) W h fo d th t f mama sth ti d bbit 1 f f i f AMI ed 2 d d gree AV blocks i 6 out f 7 imal Th i f th p t t dy t inv tig t h the thes ble ks rel t d t th ffect f AMI h li gic ystem M th c polamin (M) wa ed fo omp i

AMI s gi en by i infusi f 10 i t 0 5 g/kg/ i ECG nd he t t were ti ous ly ite d At l t two week l t th s me dogs we gi M 0 018 mg/kg i f llowed 15 25 l l t by AMI i f sion Th d g were t di d

Du i g AMI i f si he led f 2nd d g ee AV bl ks h rv d fte 3-6 i (5 i) ed l t d f 1-6 i M ws d p iod f 2 d d g AV bl cks 3-4 i ft th i j ti 1 ti g f 1 3 i The bl ks c d hortly bef si ltaneou ly with d l ping t hy rdi i f ion f AMI fter M did not 2nd degr AV bl k

Th es lt indi t that AMI may use 2nd d g ee AV bl k by i t ti i ith th perf ph l h line gi ystem

1 El E t al E J Ph rm 1 28 188 1974

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Kyöttilä J , Ylital P , El H ad Salmel P (Dept f Biomedi l bcl ces U i f Tampe Fi land) INCREASE IN THE BRAIN/PLASMA RATIO OF SOME COMPOUNDS DURING 2-METHYL 4-CHLOROPHENOXY ACETIC ACID (MCPA) INTOXICATION IN RATS

Du ing MCPA i t ication th brain/plasma rati f 14C-MCPA in ts i highly i cre ed (El H and Ylit l P A t pharma l (Kbh) 1977 41 280) W the f re compared th di t i buti f 125I um lbumin (RISA), 14C-MCPA 14C p aminobenz i cid (PABA) and 14C anti pyrine i t brain ce brospinal fl id (CSF) d mus l i mal t pretreated ith lin or t ic d f MCPA (200-500 g/kg)

Two r thre hours ft th pretre t ment the l belled t t compound wa j cted i int th aesthet ed rat and llowed t di t ibut f 10 90 in There ft CSF was llect d f 20 60 min and then th ti ues were emoved The pe ent g brain (B) CSF and muscl (M)/pla ma t f radioa ti ti f 3 5 animal were

T t compound	Dos f MCPA (g/kg) Saline B			200-250 B			500 B		
	B	CSF	M	B	CSF	M	B	CSF	M
RISA	0 8	0 2	0 9	1 4	0 3	1 3	1 3	0 4	1.5
14C MCPA	1 5	0 3	11	17	12	26	27	20	35
14C PABA	4 2	8 2	36	21	36	48	23	37	53
14C Antipy in	72	92	80	77	88	91	93	98	99

MCPA intoxi tion i re ed th distributi f 11 ompounds tudied i t the ti ue I th ca f 14C MCPA ad 14C PABA, radi cti ti the brain and CSF increased more than radio- cti iti in th ontrol ti ue

Th its indi t that MCPA t icat on impairs th b l ty f CNS t t go ze th tr b tion f some ompounds t th brain

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Komul en H and Tuomi t , J (Dept f Pharma l nd T i l , Uni f Kuopi Fi land) EFFECT OF COPPER ON THE UPTAKE AND RELEASE OF DA NA AND 5-HT IN RAT SYNAPTOSOMES IN VITRO

Many t l neurot x in CNS but t ch i s of to icity nknown Beh i l nd ment l d turb nces ften ee ithout y g patholowical damag i th brai Po ble mecha ism includ modul tion f euro tra smitt pt k and/ l f om t rag it s l ry ndi gs We hav studied th ff t f ome (a $CuCl_2$) i vit on th l iti l upt k and relea f t iti ted DA NA nd 5-HT i t c i t l o cl i ad hypo thalam vnapt ome specti ly Incub tion we i d out ith 0 l µM sub trat i K b H l t bi bon t buff pH 7 4 t 37 C and t rminated ith memb ane filtr tion IC_{50} t 2 m cubat on wer 0 8 µM (NA) 1 0 µM (DA) and 6 6 µM (5 HT) Th inhibit f DA nd 5-HT upt k wa mo competit time and aceat t on dependent nd crea d th p cub ti (5 i) f ynapt omes ith Cu^{++} M pt k wa i hibit d by l but inc ea ed by high (10 µM) on ent ti P i cub ti d ed the h bit on f NA upt k With 50 µM th high ffi ty upt k f NA wa t ll inc ea ed (t 2 l) but l t dec ed Inc ed upt k wa een l t 0°C thu t may be due t ome pa p oc pe hap bel t C^{++} i duc d lea f DA NA nd 5 HT ig ifi tly th po t eou l f om l d d vnapt som The echan sms f t f oppe ensi t b tud ed but t l t gu g that th co entra- sed d q t low and th ange po bl espe lly i N l di ease G t Ac demy f F land

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Ste bä k, F (Dep rtme f P hol gy Un ity f Kuop F land) PHOTODYNAMIC ACTION OF POLYCYCLIC HYDROCARBONS ACTIVATION AND DEACTIVATION BY ULTRAVIOLET LIGHT

Expe ime t l nc nduct on ncre sed when defi ed om ent t f be ( )py ne nd tren ty f UV ad t on l d po bly due t l t l l g f he rc nogen ncl d The purpos f h tudy t demon trat he nme t l ha d f ome nonc rc nogen h droc rbon hen omp d know nogens and multaneous radi t on th UV l gh and t ac ou und t nd g f the mol l mecha f the on

Swi 1100 femal we ad ed w th UV ha g me mum output t l nm nd pa ed on the d l k th l pol vcl hydro rbons The numbe f tumo d the morphology lua ed

The es lt f rmed the pho oc nogen ff f B( )P Al methylbe ( )an h en howed phot a f 30 ks th g f ant nc umbe f p p lloma and squamous ll oma f he d l k and Th gen ft t f d be ( )pyr howed ga f t d po bly due t d ompos on The on f the pol y l h d oc rbon hra ne Phe an h ph ne be ( )an h nd dibenz( ) thrac ne hen pa t d on k wa no ffect d by ad ion

The lt how dual ff f UV l gh abotod ompos t on f he hem l and he f rma l f non nogen bre kdow produc well pho oc rc nogen f the om pound po bly due an ac bind g f the hydroc rbon t he ll l ept lt

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Saarni H U and Pelkonen R,O (Department f Pharmacology Unive ity f Oul Finland) THE ME TABOLISM AND DNA BINDING OF BENZO( )PYRENE CATALYSED BY HUMAN PLACENTAL MICROSOMES

Aryl hydroc rbon hydroxyl se (AHH) activity i egligibl in plac ntes from nonsmoking mothers but may be levated ven 100-f ld i placenta from ci garett smok rs Because AHH i thought t be res- pon ibl f the activation f polycycli aromati hydroc rbons t mutageni and rcinogeni f rm we became int rested in mea uring the ab lity f human placent to catalyze the in litro metabolism and DNA binding f benzo( )pyrene (BP) Placental l rosomes, 3H-BP purified Salmon perm DNA and nec sary o- fact rs were ncubated and thereaft the DNA was precipitated pu ified, hydrolysed and nucleo ide- metabolit compl hromatographed by Sephad LH- 20 Incubat were tracted by thyl ac tat and met bolite patt rn measured by HPLC Phenol qui nones and one dihydrodi l (pos ibly 7 8-di l) meta- bol t were detected Both 9,10-di l and 4 5-d l were com pl tely lacking BP metabolit were bound t DNA gi ing ise t two maj peak hi h tentat vely were identified nucleoside compl f 7 8- 9 10-epoxide and 7 8-oxide and/ quinone As om- pared t binding patt rn cat lyzed by rat live l rosoma nucleo ide-metabolit peak att ibuted t 4 5-oxide and 9-phenol-4,5-oxide were lacking Re- lt demon trat that both the metabol t patt rn and nucleo de-metabolit ompl produced by pla- ental microsome i litro diff greatly from those produced by rat live l rosome The tot l binding f BP metabol te to DNA correlated gnifi antly ith both fluoromet ally mea red AHH acti ity and the amount f the di l metabolit f rmed These tu- di f rthe onfirm that t l not po bl to predi t the pecifi patt rns f DNA binding from AHH mea rement even from BP metabol te patt rn

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Ylitalonga , K Hirn M and P lkonen, O (Department t Pharma l gy Uni ity f Oulu Finland): EFFECT OF MATERNAL CIGARETTE SMOKE EXPOSURE ON FETUSES OF INBRED STRAINS OF MICE RESPONSIVE AND NONRESPONSIVE TO POLYCYCLIC HYDROCARBONS

It i known that mat rnal cig tt smoking decreases birthweight Shum t l (Pedi t Res 11, 529 1977) hav demonstrated th t f to-toxi ffect of PAH (g decrea ed weight) re intimat ly linked ith th Ah locus loco controlling the respon i en f th animal t PAH

In orde t elucid t th rol f gen t c ally-controlled re ponsi ne t PAH f to-toxic ff t f cig rett m k we expo ed pregnant C57BL/6N (esponsi) and DBA/2N (non respon i) mic t iga tt smok from day 1 to day 17 f p gnancy and tudied th outcome at day 18 The fetuses f ig rett smok exposed moth were mall than th f contr l moth The dec in bi th weight was larg in C57BL/6N (22 %) mice than i DBA/2N (17 %) mice Th d t rmin tion f AHH activity in mat rnal and f t l rgans evealed that ig rett smok i t potent indu t that time of p gnancy

Thi tudy suggests that the effects f ma ternal cig tt smoking on th f tu main ly due t the omponent than PAH becau the ffects in responsi and on spons mi were lmo t equally di tinct Back oss experime ts would be nec sary t l fy the ignificance f genetically det rmi ed espon i ene t PAH re on f th diff en between thes two t in

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Rippel T, Moilanen M-L, and Vähäkangas K. (Department f Pharmacology, University f Oul Finland) THE EFFECT OF BUTYLATED HYDROXYANISOLE ON THE METABOLISM OF BENZO(A)PYRENE IN VITRO AND IN ISOLATED PERFUSED LUNG

It has been reported that rally administered butylated hydroxyanisol (BHA) inhibits benzo( )-pyrene-induced pulmonary adenoma f rmation i ice (Spei t al 1978) and inhibit i vitro benzo( )-pyrene (BP) metabolism by rat live microsome (Rahimtul et l 1977 Spei t al 1978)

T tudy the ffects of BHA i the target rgan for BP, we added BHA and C-BP nto perfusion medium in isolated rat lung perfusions and analyzed unchanged BP, BP-metabolites and total covel t binding f BP to lung tissue BP-metabolit were isolated by thi laye chromatography fte thyl acetate xtraction All BHA-concentrations used (0 1mM 10uM and 1mM i perf sion medium) decreased the amount f unchanged BP both i the perf ion flu d and lung ti sue and increased the amount f ethyl acet te-solubl metabolites 0.1mM and 10uM BHA increased also the covalent binding f BP i to lung ti sue 1mM f BHA seemed t lightly decrease the covalent binding, but thi concentration we toxi to lung tissue

In itro 0 1mM and 1mM f BHA in incubation medium seemed also t ecrease the metabolism f BP by rat lung mi rosome The increase wa most marked i dial fraction On the contrary BHA decrea ed BP metabolism by rat liver i rosomes

Thus the ffects f BHA seem to be diff rent i lung ti sue compared to live Thi tudy indi ates that both metabolism f BP and covalent binding f BP to lung tissue re increased by BHA

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Ahotupa M and Aiti , A (Department f Physiology University f Turku Finland) INDUCTION OF XENOBIOTIC BIOTRANSFORMATION REACTIONS BY POLYCHLORINATED NAPHTHALENES IN RAT LIVER

Commercial mixtures of polychlorinated naphthalenes (PCN) (Halowax) were potent inducers of enzymes catalysing drug hydroxylation or glucuronidation The trongest inducer was Halowax 1051 the PCN with the highest chlorine content (70 wt %) A ingle i p dose (100 mg/kg) of it 7 days before assaying the enzyme activitie enhanced thoxycoumarin deethylation 10-f ld benzo( )pyrene hydroxyl tion 2 5 f ld and glucuronidation f 4-methylumbel lif rone and 2-aminophenol 3-f ld The inductions wer dose-dependent; 10 mg/kg was suffi ient to enhance hydroxylation and glucuroni dation reactions The hydroxyl tion and glucuronidation reactions achieved maximal rates in one week they were till above control level after one month

The ctivities f epoxide hyd tase and glutathione S-transferase were not changed aft PCN-treatment

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ACTA PHYSIOLOGICA SCANDINAVICA
Supplementum 474

ON INTEGRATIVE MOTOR FUNCTIONS IN THE CAT'S SPINAL CORD

by

HANS FORSSBERG

STOCKHOLM 1979

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FROM DEPARTMENT OF PHYSIOLOGY III
KAROLINSKA INSTITUTET STOCKHOLM SWEDEN

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CONTENTS

This thesis mainly constitutes a summary of the following articles

I FORSSBERG H GRILLNER S & HALBERTSMA J 1979 The locomotion of the low spinal cat 1 Coordination within a hindlimb Acta Physiol Scand In press

II FORSSBERG H., GRILLNER S HALBERTSMA J & ROSSIGNOL S 1979 The locomotion of the low spinal cat 2 Interlimb coordination Acta Physiol Scand In press

III FORSSBERG H GRILLNER S & SJOSTROM A 1974 Tactile placing reactions in chronic spinal kittens Acta Physiol Scand 92 114 120

IV FORSSBERG H GRILLNER S & ROSSINGOL S 1977 Phasic gain control of reflexes from the dorsum of the paw during spinal locomotion Brain Res 132 121 139

V FORSSBERG H 1979 Stumbling corrective reaction A phase dependent compensatory reaction during locomotion J Neurophysiol 42 936 953

VI ANDERSSON O FORSSBERG H GRILLNER S & LINDQUIST M 1978 Phasic gain control of the transmission in cutaneous reflex pathways to motoneurones during fictive locomotion. Brain Res 149 503 507

The papers are referred to by their Roman numerals in the text

GENERAL INTRODUCTION

Born into a hostile environment an animal must immediately be able to execute a number of functions in order to survive Breathing screaming for food opening the mouth swallowing and eliminating body wastes are examples of innate motor behaviours that are specifically and genetically encoded into the nervous system for each species (see Tinbergen 1951 Hinde 1966 Eibel Eibesfeldt 1970 Bentley & Konishi 1978) Later in life similar innate neural programs control the behaviour of the animal in a number of important activities such as propulsion reproduction feeding infants and escaping enemies It is primarily in the phylogenetically more evolved species that learned motor behaviours play a significant role during the daily life (see Thorpe 1964 Harlow & Harlow 1962 Wilson 1975)

A complete neural explanation of these behaviours involves several factors A description is needed of the organization of the basic neural networks that are programmed to produce the specific motor task and of the central control (e g volition) of these networks The sensory elements that can initiate activity in the networks and the effector organs that execute the movements should also be identified These problems can be properly solved in simple motor behaviours such as certain reflexes in mammals or isolated motor functions in invertebrates (see Bentley & Konishi 1978) In the more complex mammalian behaviours the number of neural elements increases to an extreme level

This summary is limited to the study of the neural control of some innate motor functions in the cat The studies are focused on the generation of the basic locomotor activity that supports an animal and propels it forward and also on spinal mechanisms involved in the adaptation of the locomotor movements to

the environment For more extensive reviews on the neural control of the locomotion see Grillner (1976 1979b Shik and Orlovsky (1976) and Wetzel & Stuart (1976)

METHODOLOGICAL CONSIDERATIONS

To understand the neural control of a certain motor behaviour it is important to know the contribution of different parts of the central nervous system as well as the mechanics of the skeleto-muscular system The organization of the control can be divided into several levels e g (i) the mechanisms of the individual neurones (ii) the organization of neurones into net works that generate certain patterns of activity (iii) the function of different receptors and interacting reflex pathways (iv) The final movement depends on the biodynamics of e g the limb and of the relevant muscles

It is almost impossible for the same investigator to master all different levels We have attempted to study the effect of the integration of the activity from all these levels by means of recordings of the muscular activity the movements and the forces but also to explore some neural mechanisms (see above)

Chronic spinal cats were used in papers I IV They were subjected to a transection of the spinal cord (Th_{12}) 1 2 weeks after birth and thereafter nursed and trained carefully to about 6 month of age The locomotion of the spinal cats on a treadmill was studied in papers I and II Tactile placing reactions as described by Bard (1933) were elicited in paper III with strain sensitive devices recording contact and landing of the foot Stumble corrective reactions elicited during treadmill loco motion were studied in chronic spinal cats (paper IV) and in intact cats (paper V) by tactile stimuli applied to the dorsum of the foot during different phases of the stepcycle

In paper VI intracellular recording technique was used during fictive locomotion (Edgerton et al 1976) Injection of DOPA and Nialamide in acutely spinalized and curarized cats could initiate rhythmic locomotor activity (Grillner & Zangger

1975) In such preparations it was possible to record from motoneurones and study the mechanisms of phasic gating during ongoing central locomotor activity without any phasic influence from the periphery

Muscular activity In papers I V we used pairs of insulated copper wires (Ø 0 1 mm) with 1 mm uninsulated at the tip inserted into desired muscles with hypodermic needles (cf Engberg & Lundberg 1969) Electrode positioning was verified with stimulation between each EMG-electrode and a ground electrode and simultaneous palpation of the contracting muscles The EMGs were preamplified low (5000 Hz) and high (50 Hz) pass filtered and usually rectified and filtered (see Gottlieb & Agarwal 1970) to study the total envelope of activity In the later experiment (paper I and II) the rectified and filtered EMG signals were fed via an Analogue Digital Converter (sampling rate 312 Hz) to a Minicomputer (HP 21 MX) for further analysis

Movements The introduction of the cinemathography was a great step forward in the study of motor behaviours The movements of the entire limb and the angular movements of the joints could be analyzed (see Marey 1895 Engberg & Lundberg 1969 Goslow et al 1973 and Philippson 1905) In the beginning of our studies (papers III and IV and part of I and II) we used a 16 mm film camera with a speed of 64 frames/s The position of the joints of the hindlimbs was measured on a graphic tablet connected to a desk calculator (HP 9830A) from which the angular movements of the joints could be calculated and plotted as well as the consecutive positions of the limb (stick diagrams) In later studies (papers I II and V) the movements were recorded by a Selspot System in which infrared light emitting-diodes were glued to the skin over the joints These diodes were detected by a special camera (156 Hz) from which the x y coordinates of the diodes could be fed into a minicomputer (HP 21 MX) and stored on magnetic tape Two cameras allowed simultaneous recording from both hindlimbs (paper II) After standard data treatment (filtering corrections) the angular movements of the joints and the movements of the limb could be directly plotted on a graphic terminal (Tektronix 4010-1)

Forces The forces produced by a muscle during ongoing movements have usually been indirectly calculated from movement recordings (film) recordings of the reaction forces (force plate) and with the knowledge of the insertion of the muscles and the mass and center of gravity of each limb segment (Manter 1938 Bernstein 1967 Grillner 1972) Recently it has been possible to attach small force transducers to muscle tendons and directly record the tension from individual muscles (Walmsley 1978)

In our work (paper I) we used a forceplate (Kistler 9261A) simply to record the vertical and horizontal forces produced by the limb during the support phase

ABBREVIATIONS

The following abbreviations are used Flexion phase F first second and third extension phase E_1 E_2 and E_3 M Flexor Digitorum Brevis FDB M Flexor Digitorum Longus FDL; M Gastrocnemius Lateralis LG M Gastrocnemius Medialis MG M Soleus Sol M Plantaris Pl M Quadriceps (Vastus Lateralis) Q M Gluteus Medius GM M Semitendinosus St; M Posterior Biceps PB M Tibialis Anterior TA M Iliopsoas IP M Sartorius Sart M Extensor Digitorum Brevis EDB Electromyography EMG Standard Deviation SD; Standard Error SE

RESULTS AND DISCUSSIONS

A SPINAL LOCOMOTION

In 1874 Eichhorst and Naunyn and Freusberg reported that the lumbar spinal cord isolated from supraspinal structures could evoke rhythmical sequences of flexion and extension of the hind limbs which later was called spinal stepping (see Sherrington 1910) Philippson (1905) analyzed films from spinal stepping in dogs and emphasized the similarity to normal walking This similarity was however not generally accepted and it was argued that the forelimbs carried the body weight and propelled the animals forward (Magnus 1924 ten Cate 1940) Sherrington (1910) seemed to have accepted spinal stepping as the basis for normal walking but he emphasized the difference between spinal stepping and the "walking of decerebrate cats He pointed to the weakness of the extension phase in the spinal animals and suggested that a supraspinal contribution was necessary Later investigators (Kellog et al 1946 Shurrager & Dykman 1951 ten Cate 1962 1964 Stelzner et al 1975) have improved the walking movements in different animals using different training and manipulative procedures

Directly after a transection of the spinal cord there is practically no reflex activity caudal to the lesion (spinal shock see Sherrington 1947) Depending on the age and species of the animal it lasted some hours up to several days until locomotor like activity could be induced after the spinal transection (Freusberg 1874 Sherrington 1910 Shurrager & Dykman 1951 Stelzner et al 1975) Noradrenergic precursors (DOPA) or receptor stimulators (Clonidine) could however release spinal stepping or treadmill walking in adult animals immediately after an acute transection of the spinal cord (Grillner 1969 Budakova

1973 Forssberg & Grillner 1973) These walking movements were analyzed in some detail with regard to the temporal pattern of the EMG activity and the adaptation to the speed of the tread mill belt

a) Generation of cyclic locomotor activity in a single hindlimb

Although spinal stepping and spinal walking are well documented in the literature (see above) few attempts have been made to define how complete the locomotor pattern is which the spinal cord can produce without supraspinal influence In papers I and II we have attempted to do such a study by analyzing the movements the muscular activity and to some extent the reaction forces during locomotion of chronic spinal cats

1 Movements Kittens subjected to a transection of the spinal cord at low thoracic level (Th_{12}) 1 2 weeks after birth showed alternating flexion and extension movements when lifted in the air 1 2 days after the operation with the spine held vertically When the animal was placed on a treadmill the alternating movements changed character and the hindlimbs walked with a swing phase in which the foot was lifted in the air and the limb moved forward and with a support phase in which the foot contacted the belt and the limb moved backward in relation to the body As the kittens grew older the walking improved and based on the angular movement in the hip knee and ankle joints the swing phase could be divided into a flexion phase (F) during which the foot was lifted with a flexion in all joints and a first extension phase (E_1) when the foot was lowered by an extension in knee and ankle during continuing hip flexion The hip started to extend just prior to foot placement and extended during the whole support phase but due to the movements in knee and ankle the support phase could be divided into a second extension phase (E_2) in which knee and ankle flexed due to the sudden load and a third extension phase (E_3) in which all joints extended together (see Fig 1,Philippson 1905) Similar angular movements have been recorded from locomotion in intact cats (Engberg & Lundberg 1969 Goslow et al 1973) Although the basic movements of the spinal cats were similar to those during normal locomotion there were some deficits Unless prevented from falling the

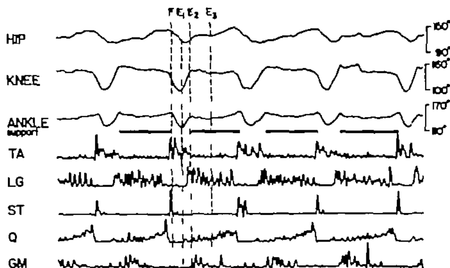


Fig 1 The angul movement f the hip kn and ankl (at the t p) from a hroni pinal cat walking on a tr admill (0 20 m/s) ar pl tted togethe with the imultaneously eco ded EMGs of TA LG St Q and GM. The onset f the flexion and exten ion phases based on the movement of the ankl i indi ated with v rtical lines The upport phases ar p esented by schemati blocks The time calibration i on the bottom i econds (l rg ba) with 200 ms ubdivisions (small bars)

hind quarters would fall to one side or the other (equilibrium control) The extension during the end of the support phase (E_3) was usually too weak especially during slow walk and the knee flexed often before the extensor activity had ceased (see Fig 1). There was also usually a narrow" walk in which the feet could hook onto each other presumably due to an increased tonus in the adductor muscles of the hip or to a relative weakness in the abductor muscles

2 Muscular activity In general flexor muscles are active during the swing phase and extensor muscles during the support phase but depending on the joint at which the muscle acts the acti ity has a precise onset and termination and a characteristic envelope Several EMG-studies during locomotion have been per-

formed on intact cats and dogs some reporting both duration and intensity of the locomotor bursts (Engberg 1964 Engberg & Lundberg 1969 Gambarian et al 1971 Tokuriki 1973; dog Smith et al 1977 Walmsley et al 1978 see also paper I) others only the duration (Wentink 1976 dog Rasmussen 1978) Below the pattern of muscular activity during spinal locomotion will be compared to that during normal locomotion (see Fig 2) Unless there are discrepancies in the results from the different EMG-studies of intact locomotion no references will be given

The muscles are referred to as extensor or flexor muscles according to Sherrington s (1910)definition in which extensor muscles counteract gravity during standing and are activated by the crossed extensor reflex and where flexor muscles are participating in the flexor reflex

Extensor muscles of both intact and spinal cats are active approximately from the middle of E_1 (end of swing) until near the end of E_3 (end of support) Engberg & Lundberg (1969) emphasized that the muscles became active before foot placement and that the activity consequently was not induced by reflex effects due to ground contact Extensors of the toes (FDB FDL) and the ankle (LG MG Sol Pl) have an intense onset with an early peak followed by a slow decline and a smooth cessation Corresponding muscles of spinal cats (FDL LG Sol) had the same duration and envelope of activity at corresponding velocities (see Fig 2) The knee extensor (Q) can show two different patterns both seen in intact and in spinal cats Either there can be a continuous burst with a smooth initiation and a successive increase of activity until the very end of E_3 where it ends sharply (see Fig 2) or there can be two separate bursts of which a smaller one occurs during early E_1 and a larger and square shaped one is present during E_2 E_3 (Engberg & Lundberg 1969 see Fig 4 in paper I) Both patterns could be seen in the same intact or spinal cat the former predominantly at slow walks The hip extensor (GM) had a more symmetric form of activity in both preparations with a smooth onset and termination and a peak during the beginning or middle of the support phase (Fig 2)

Flexor muscles are not as uniformly activated as the extensors The knee flexors (St PB) are activated twice during each step-

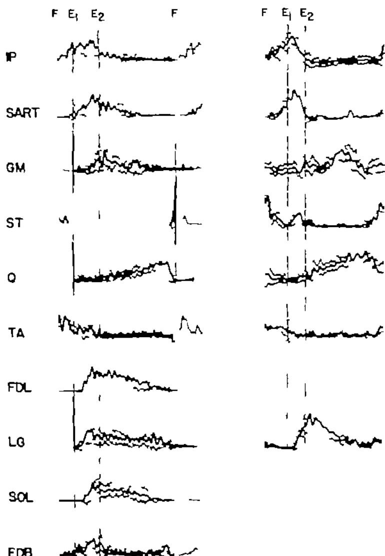


Fig 2 Ave aged EMGs from pinal and intact cats The rectified and filt red EMGs from th muscles indicated (ee Abbreviations) ar av aged after normalization f the step cycl du tion The mean is pl tted as continuous lin and the Standard deviation (SD) as dotted lines Th recordings re from diff rent cats (hron pin n 2; intact n 2) and from diff rent experimental sessions but are till comparable ince all cycl durations re about 800 ms (±20 ms) and as the ons t of the av raging is trig- ge ed by the movement of the ankl j i t (ee Methods in paper I) The ons t of the flexion and extension phases e marked as vertical lines The numb of averaged locomotor b rst vary between 8 and 12

cycle in intact animals once with a short and intense burst at the end of E_3 and beginning of F and a second time with a smaller burst during E_1 E_2 In the spinal cat the first burst was always present while the second burst could be weaker or absent especially at slow walks (see Fig 2) The second burst was however seen in all spinal cats during faster walk although less prominent than normal The ankle flexor (TA) has one burst during intact walk that starts somewhat later and is smoother than the first knee flexor burst In our study (see Fig 2) as in Wentink (1976) Tokuriki (1973) and Gambarian et al (1971) the plateau like activity outlasted that of the knee flexor and ceased rather suddenly At the same time the hip flexor activity started to decline with a much slower time course On the contrary Rasmussen et al (1978) reported TA activity until the beginning of support (longer than hip activity) and Engberg & Lundberg (1969) reported the activity of the ankle flexor to be similar to that of the knee flexor (see however Fig 22 in Engberg 1964) The hip flexors (IP Sart) have a gradual onset at about the same time as the ankle flexor during intact walk The activity differs however from that of the ankle flexor and has a pronounced late peak and a longer overall duration (see Fig Engberg & Lundberg 1969 Tokuriki 1973 Wentink 1976) In the spinal cats the ankle flexor (TA) usually had an intense onset simultaneously with the knee flexor The hip flexors (IP Sart) started smoother and somewhat later (see Fig 2) Both hip and ankle flexors were active longer than the knee flexor The ankle flexor had a successive decrease of activity from the intense onset until the middle of E_1 while the activity of the hip flexors were increased until a peak during mid E_1 and then decreased (see Fig 2) During intact walking the short toe flexor (EDB) has a short intense burst during E_1 and a residual activity during the larger part of the support phase A similar pattern was found in the spinal cats (see Fig 2)

Conclusion The EMG-pattern from individual muscles during spinal locomotion shows striking similarities to that of intact walking cats This implies that the spinal cord with intact afferents can produce a detailed pattern of muscular activity

The specific onset and termination of the activity as well as the characteristic envelope of the locomotor burst is programmed for each individual muscle There are however some smaller differences as the usual absence of the second St burst at lower speeds This loss is presumably due to a low excitability level of this part of the network, and not to a loss of the neural components that induce the burst as it could be seen at higher velocities The more simultaneous activation of knee (St) and ankle flexors (TA) was another difference It is however noteworthy that a time delay similar to that during normal walk has been found during fictive locomotion (Grillner & Zangger 1979)

3 Force During the support phase the extensor muscles shall support the body and propel it forward The extensors of the hindlimbs of the spinal cats could carry the weight of the hind quarters during locomotion although the cats needed assistance to maintain equilibrium (see Fig 6 in paper I) The knee did however not manage to extend during the end of E_3 when the maximal force was required (see below) but yielded under the load (see above) This yield could be due to either insufficient muscular force or to a required torque that was higher than normal The forwardly directed force from the hindlimbs mainly required from the hip extensors was clearly too weak to bring the animals forward alone It was necessary to extend the hips to initiate walk unless an unspecific stimulus was used (see below) But also when the cats were stimulated e g squeezing the tail the forward force was very weak (see Fig 6 in paper I)

The force required from the extensor muscles during a support phase in normal cats has been calculated from combined film and force plate recordings (Manter 1938 Grillner 1972) and recently also recorded from force transducers attached to muscle tendons (Walmsley et al 1978) In the ankle and the hip the maximal torque is required in the beginning of the support while in the knee it is needed in the end (Manter 1938 Zomlefer unpubl) The relative envelope of the locomotor bursts in spinal as well as in the intact cats corresponds surprisingly well to the required forces at the different joints during the different phases of support (as calculated in intact cats)(see Fig 2)

The flexor muscles have to both stop and reverse the direc tion of the movement of the limb The hip muscles have an impor tant function in moving the limb forward during the largest part of the swing phase The main task of the more distal flexors beside stopping the extension is to flex the limb in the beginning of swing in order to bring the mass of the limb closer to the pivot point It is therefore functional with the early and short activity in the distal flexors and the later main ac tivity in the hip flexors (cf Engberg & Lundberg 1969) The short burst of the knee flexors (St or PB) is presumably due to the double function of the muscles who also extend the hip They would counteract the flexion if active in later parts of the swing phase when the hip is flexed (Grillner 1975)

4 Speed adjustments When the speed of locomotion increases in intact cats the number of stepcycles per second increases markedly At the same time there is at least at lower speeds a substantial increase of the support length i e the distance the animal moves during each support phase (see Grillner 1975 paper I) The reduction of the cycle duration is largely due to a shortening of the support phase with only a small reduction of the swing phase (mainly E_1) (Goslow et al 1973 Halbertsma 1976) The locomotion of the spinal cats followed within some limits the speed of the treadmill belt Similar adjustments of the support length and of the phases did occur as during intact locomotion at faster walks The duration of the extensor bursts shortened in parallel with the reduction of the support phase with maintained total muscular activity due to an increased overall intensity A prolonged and enhanced flexor activity succeed ed to breake the enhanced extension and to slightly shorten the swing phase (see Fig 2 in paper I) Hence there are appropriate speed adjustments of the spinal mechanisms that produce the locomotor activity

<u>5</u> Central generation Throughout the animal kingdom it has been shown that a variety of innate motor behaviours such as breathing (Bradley et al 1975) mastication and feeding (Dellow & Lund 1971 Miller 1972 Maynard 1972 Selverston 1976) and locomotion (Wilson 1961 Wiersma & Ikeda 1964 Székely et al

1969 Pearson 1972 Grillner & Zangger 1975 Grillner et al 1976) are coordinated by means of central neural networks It has been demonstrated that a significant part of the networks that generate locomotion in the cat are located in the spinal cord as spinal deafferented (Brown 1911 1914) or curarized preparations (Jankowska et al 1967a b Grillner & Zangger 1974 1979 Viala & Buser 1969 rabbit Vidal et al 1979 rabbit) can produce alternating flexor and extensor bursts with some retained temporal specificity between different type of muscles (Grillner & Zangger 1979 see also Fig 2 3 in Vidal et al 1979) Paper I demonstrates that spinal cats with intact afferents can produce a replica of the intact locomotion and studies on decerebrate and decorticate cats that the detailed timing of the different locomotor bursts can be generated without phasic feedback related to the movements (Grillner & Zangger 1975 (and unpubl) Perret & Cabelguen 1975)

6 Peripheral control of the locomotor generator The fact that locomotion was induced when a spinal cat was placed on a moving treadmill belt and adjusted to the velocity of the belt (see above) implies that peripheral influences are exerted on the locomotor generator (paper I) Sherrington (1910) noted that spinal stepping occurred when the animals were held with vertical spine and he found that hip extension was sufficient to initiate stepping while passive extension of ankle or knee alone or together did not suffice Orlovsky (1972) showed during on going locomotion in mesencephalic cats that an arrest or an acceleration of the hip flexion prolonged or reduced the duration of flexion of the entire limb

The locomotion of the hindlimbs of the spinal cats was initiated by extension of the hip both when the animal was held over a treadmill with the feet on the belt and when the animal was standing on the ground and started to walk with the forelimbs (Budakova 1973 Forssberg & Grillner 1973 paper I) If during treadmill locomotion one hindlimb was stopped during the support phase the extensor activity was maintained until the limb was brought backward to the position of the hip where flexion usually occurres during normal locomotion At this position the extensor activity suddenly ceased and the limb briskly flexed

(Rossignol et al 1976 Grillner & Rossignol 1978) Similarly flexed hip position was found to promote extensor activity during fictive locomotion (Andersson et al 1978) These effects where the position of the hip influences the locomotor generator to switch from extensor to flexor activity (Rossignol et al 1976 Grillner & Rossignol 1978 Andersson et al 1978) or to initiate extensor activity (Andersson et al 1978 Grillner 1979a cf Orlovsky 1972) has been regarded as a position dependent negative feedback (Grillner 1979a) Andersson et al (1978) have however also described a directional sensitive mechanism *in which small movements of the hip entrained the rhythmic activity during* fictive locomotion (Grillner 1979a; cf Grillner & Wallén 1977)

The position dependent control regulating the amplitude of hip extension during locomotion, will control the duration of the support phase during different speeds But what induces the enhancement and prolongation of the flexor bursts that occur at increasing velocity (see paper I)? It seems probable that events in the ipsilateral limb occurring prior to the flexor activity would be the determining factor such as the duration of the extensor activity or the velocity of extension It is also possible that an increased rate of unspecific stimuli that are known to excite the locomotor activity (cf Grillner 1975 Grillner & Zangger 1979 paper I cf Sherrington 1910) could play some role in the speed adjustments

Pearson & Duysens (1976) found that an extensive load applied to the ankle extensor muscles could prevent the switch from extensor to flexor activity During normal locomotion the torque around the ankle decreases at the end of support (see force above) An increased load on the ankle extensors would imply that the limb is not brought backward or that there is an increased load on the limb (cf Grillner 1979a) In both situations it would be meaningful with a prolonged support and a delayed swing

b) Interlimb coordination

During normal quadrupedal locomotion the movement of each limb has to be coordinated with the movement of the other three limbs An animal can select among a number of patterns depend

1969 Pearson 1972 Grillner & Zangger 1975 Grillner et al 1976) are coordinated by means of central neural networks It has been demonstrated that a significant part of the networks that generate locomotion in the cat are located in the spinal cord as spinal deafferented (Brown 1911 1914) or curarized pre parations (Jankowska et al 1967a b Grillner & Zangger 1974 1979 Viala & Buser 1969 rabbit Vidal et al 1979 rabbit) can produce alternating flexor and extensor bursts with some retained temporal specificity between different types of muscles (Grillner & Zangger 1979 see also Fig 2 3 in Vidal et al 1979) Paper I demonstrates that spinal cats with intact affe rents can produce a replica of the intact locomotion and studies on decerebrate and decorticate cats that the detailed timing of the different locomotor bursts can be generated without phasic feedback related to the movements (Grillner & Zangger 1975 (and unpubl) Perret & Cabelguen 1975)

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between the belts the fast limb could perform a multiple of steps (2-4) during one stepcycle of the slow limb (Kulagin & Shik 1970 paper II cf von Holst 1939) The duration of the stepcycles of the fast limb as well as the flexion and extension phases differed markedly depending on the phase of locomotor activity of the other limb (paper II)

These findings imply that there is a potent interaction between the locomotor generators (see von Holst 1939 Arshavsky et al 1965 Shik & Orlovsky 1965 Gelfand et al 1971 Kulagin & Shik 1970 Stein 1976 Grillner 1976 1979b Grillner & Zangger 1979) that can influence each other particularly during the swing phase to generate the same or multiple rhythms though the peripheral influences differ Grillner and Zangger (1979) showed from experiments with fictive locomotion that the locomotor activity on the two sides were properly coordinated which implies a pure interaction between the generators It is however important to realize that the peripheral influence on one side also directly may influence the contralateral generator It can thus not be taken for granted that the effects are only due to interaction between the two independent generators

Disregarding the detailed neural mechanism of the interaction an interesting implication is that the brain does actually not need to concern itself with adjustments of the hindlimbs when the animal is turning It would be sufficient for the front part to turn and the hindlimbs would by the diverging peripheral influences from the two hindlimbs and by the interaction of the generators make the appropriate changes (paper II cf Grillner 1979b)

c) Concluding remarks

Neural networks within the spinal cord with intact reflex interaction can generate locomotion in the chronic spinal cat The spinal mechanisms can even reproduce subtle details of walking movements and of signals to the muscles (EMGs) of the intact walking cat A central locomotor generator produces specific locomotor activity Due to peripheral influence the generator adjusts the locomotor activity to different speeds The activity of the two limbs is properly coordinated by mutual interaction of the two locomotor generators With such a comp-

lete organization in the spinal cord it would be sufficient for supraspinal centers simply to control the excitability level of the neural circuits (cf Grillner 1975) An increased tonic facilitation could increase the activity of the generators which alone could make the required adjustments Such spinal circuits may indeed operate also in the normal and adult cat as locomotion can be induced in acute spinal cats after injection of DOPA or Clonidine (Jankowska et al 1967a b Grillner 1969 Budakova 1973 Forssberg & Grillner 1973)

B PLACING STEPPING AND HOPPING REACTIONS

a) The reactions

Magnus (1924) Rademaker (1931) and Bard (1933) described several placing and hopping reactions that were assumed to aid animals in placing their feet Visual placing occurred when the head approached an object and consisted of a flexion of the limb and a subsequent placing of the foot on the top of the object Exteroceptive placing could be elicited from the chin the vibrissae the tail or the dorsum of the foot. If an animal was held in the air with its limbs hanging a light contact of the dorsum of the foot with the edge of a table would elicit a tactile placing reaction in which the foot was immediately withdrawn and accurately placed sole down close to the edge of the table (Bard 1933) Touching the medial or lateral aspect of the foot resulted in a placement in a more medial or lateral position (see Amassian et al 1972) A stimulus to one foot could induce bilateral reactions (Bard 1938) A more extensive contact that would include activation of proprioceptors elicited a reaction similar to tactile placing If the body of a standing animal was displaced forward backward or sideways in relation to the feet a new and more appropriate limb position could be achieved by making a step (hop) in a direction that resulted in a more stable stance If all limbs (or two) were free it would execute consecutive steps If standing on one limb (other limbs suspended) it could hop by a flexion and a subsequent extension in the direction of the displacement (Rademaker 1931 Bard 1933)

A hop correction for a forward movement is thus very similar to the placing evoked from a displacement of the dorsum of the foot as proprioceptors from the proximal part of the limb (hip) are contributing to both reactions (Rademaker and Hoogerwerf 1930 Bard 1938) Similar responses may thus be evoked by different receptor systems even if they are modifiable in relation to the site of the visual or exteroceptive stimuli or the direction of the displacement

Tacile placing has been studied more than the other reactions The flexor muscles become activated 20-40 ms after foot contact and the succeeding extensor activity evokes placing of the foot 180 550 ms after contact (Amassian et al 1972 Norrsell and Lundberg in Lundberg 1973)

b) Basic organization in the spinal cord

Stepping hopping proprioceptive and tactile placing reactions could all be elicited in animals subjected to a transection of the spinal cord at lower thoracic level (Forssberg et al in Grillner 1973 paper III Stelzner et al 1975 rat see also Lundberg 1973) *A very light touch by a fine brush only deflecting the hairs on the dorsum was sufficient to elicit a tactile placing reaction in the hindlimbs which in all aspects correponded to Bard s original description (1933)* Also lateral or medial placing (see above) could be evoked (paper III) In contrast to what was thought earlier (Bard 1933 1938 Amassian et al 1972) these corrective reactions of the hindlimbs can thus be generated by spinal reflex networks

The striking similarity of the different reactions suggest that the same neuronal network is used although it becomes activated by different types of receptors A seemingly less likely alternative is that each different receptor system should have its private placing network The flexion extension movement resembles a single step (see Bogen and Campbell 1962) The basic locomotor activity (flexion-extension) is generated by a central spinal network which can be influenced from the periphery for instance by hip movements (see spinal locomotion above) It is possible that different correcting reactions at least in part utilize the central locomotor network to induce a step (paper I)

The hip extension that may initiate locomotion on a moving treadmill is the same kind of stimulus that can evoke a hopping or stepping reaction in the forward direction Spinal cats can not only produce a backward hop or step but also actually walk backwards on a treadmill (Forssberg Grillner & Halbertsma unpublished) Furthermore tactile placing can generally be evoked with lower threshold and brisker reactions just after the chronic spinal cats have been walking (paper III) What about the medial lateral placing reactions? A basic flexion-extension movement

is clearly involved that may be caused by the locomotor generator (see preceding paragraph) but a direction specific reflex effect must also be added Such an effect could simply be due to additional excitation to abductor (lateral placing) or adductor (medial placing) motoneurones that in any event are activated by the locomotor generator

c) Supraspinal control

Bard and co-workers performed a series of studies in different species on placing and hopping reactions in the beginning of the 1930 s which have influenced the litterature ever since Hopping and proprioceptive placing but not tactile placing could be elicited in reptiles (alligator lizard) (Bard et al 1932 see Bard 1938) Both tactile and proprioceptive reactions could be evoked in rat or rabbit but tactile placing was not as well developed as in the cat or the monkey After decortication it was abolished in all species while the proprioceptive reactions remained in rat rabbit and in the reptiles (Bard et al 1932 Bard 1933 Brooks 1933 Brooks & Woolsey 1936 Woolsey & Bard 1936 Bard 1938) Tactile placing of the cat was further shown to be dependent on a restricted area of the contralateral sensori-motor cortex and was depressed if this area was removed but remained when the entire cortex except this part was taken away (Bard 1933) On the basis of these experiments it was suggested (see Bard 1933 1938) (i) that the neural mechanisms of the reactions migrated to higher levels during phylogeny (ii) that the tactile reactions were more advanced than the proprioceptive ones and organized on a higher level and (iii) that a localized area of cortex was an essential part of the tactile reactions in higher animals (cat monkey)

In experiments on monkeys Bard found that hopping reactions depressed after a contralateral ablation of the cerebral cortex reappeared when the ipsilateral cortex was also removed After this finding he modified his suggestion concerning the proprioceptive reactions and pointed to the possibility that subcortical centers could induce the reactions although a tonic control was exerted from cortex (suggestion of McCulloch in Bard 1938) Later investigators also found a reappearance of tactile placing after an ipsilateral decortication following a contralateral one (Denny-Brown & Chambers 1958 Bogen & Campbell 1962)

Tactile reactions have also been elicited in extensively neo decorticated cats and in acutely decerebrated cats after an in jection of d amphetamin (Maling & Acheson 1946 Meyer et al 1963) and in chronic spinal animals (paper III Stelzner et al 1975)

Since a spinal network has been shown to generate tactile placing in the hindlimbs (see above) the original view sugges ting long loop reflex (including even cortex) cannot be the only mechanism that produces the reaction (see Fig 3 alternative 1)

Independently of its mechanism the depression of the reac tions following a contralateral ablation of the sensori-motor cortex (Bard 1933) depends on the pyramidal tract (corticospinal tract) as a depression occurs also after a pyramidotomy (Tower 1940 Gilman & Marco 1971) The reappearance of the reactions following an ipsilateral hemi decortication (Bard 1938 Denny Brown & Chambers 1958 Bogen & Campbell 1962) must result from a net facilitation of the circuits that are responsible in the decorticated state They may be spinal but additional supra spinal loops cannot be excluded (see below)

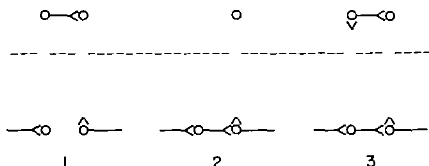


Fig 3 Thr theo tically different organizations f the neural reflex netwo k that may produc tactil placi g One int rn urone in the diagram may represent a chain 1) A long loop reflex mediated vi supraspinal structures 2) A spinal basi n twork toni cally influenced from highe level 3) A long loop reflex superimposed on the spinal reflex n twork

The ipsilateral Nucleus Interpositus also seems to facilitate tactile placing (Snider 1940 Chambers 1953 Chambers & Sprague 1955) presumably via the red nucleus (cf Pompeiano 1967 Amassian et al 1972) The intermediate part of the cerebellar anterior lobe has an inhibitory influence on Nucleus Interpositus and a selective lesion enhances placing (Pompeiano 1967) Ipsilateral lesions of Nucleus Reticularis Lateralis or an ipsilateral denervation of the neck (C_1-C_3) abolish the placing reactions presumably due to effects on nucleus interpositus (Corvaja et al 1977 Manzoni et al 1979) Information from several central nervous structures can thus via cerebellar circuits influence the performance of the placing reactions

Lesions of an uncrossed pathway in the mediodorsal part of the lateral funicle abolishes tactile placing (Lundberg & Norrsell 1960) Activity related to placing reactions occurs in the ventrolateral nucleus of the contralateral thalamus (Amassian et al 1972 Massion & Smith 1973 1974) Finally an ablation of the post central gyrus abolishes placing of the contralateral limb as well as crossed placing of the untouched limb (Bard 1938 Lumley 1973) Theoretically the supraspinal control can be exerted in two different ways (i) by tonic facilitation or inhibition (alternative 2 in Fig 3), or (ii) by long loop reflexes superimposed on the spinal reflex networks (alternative 3 in Fig 3) The ascending activity could constitute an afferent link in a long loop (alternative 3) but the depression of the reactions seen after the lesions can as well be due to indirect tonic effects on presumed cortical or subcortical centers that control the spinal networks (i e alternative 2) There are no possibility at present to decide between the remaining alternatives (2 or 3 in Fig 3) The supraspinal centers in intact cats can nevertheless control the reaction and depress it when it is not useful (see Amassian et al 1972)

It is noteworthy that several authors influenced by Bard s original reports still discuss placing reactions as cortical reflexes (Henneman 1974) or as learned responses dependent on the integrity of motor cortex (Roberts 1978; pp 173 and p 296) or use the reactions to study higher level control of movement (Amassian et al 1972 1977) or development of the corticospinal tract (Donatelle 1978) although it has long been known that sub-

cortical centers can generate the reactions (Bard 1938 Maling & Acheson 1946 Denny Brown & Chambers 1958 Bogen & Campbell 1962 Meyer et al 1963 Stelzner et al 1975 paper III)

d) Development of the control of the tactile placing reaction

Tactile placing in the rat develops 7 18 days after birth at the same time as the pyramidal tract grows into the spinal cord (Tang 1935 Hicks & D Amato 1975 Donatelle 1978) When a hemispherectomy was performed at birth the tactile placing developed as usual (from the end of the first week) until around the 17th day when the responses ceased contralateral to the lesion If however the motor cortex was removed bilaterally at birth no later impairment occurred (see Hicks & D Amato 1975) A mid thoracic lesion of the rat spinal cord prior to the 15th post natal day neither disturbed tactile placing nor the existing locomotion while it later induced severe disturbances and could abolish the reactions altogether (Stelzner et al 1975 Weber & telzner 1977 see also Bignall 1974)

After a unilateral removal of sensori-motor cortex in kit tens before the fourth postnatal week tactile placing first returned but was subsequently lost at one to two months of age (Amassian & Ross 1973) Cooling of the cortex (kittens) had no effect during the first post natal week but abolished placing after the end of the second week (Amassian 1976)

There is thus an initial period during which a blockage of the supraspinal control does not affect the performance of the tactile placing reactions or locomotion During this period spi nal networks must be responsible to a significant degree Afterwards these networks somehow become dependent on supraspinal structures The actual mechanism is unknown It should be noted however that the motor reactions can be activated in adult ani mals after lesions at different levels and that the networks thus still are intact (Maling & Acheson 1946 Meyer et al 1963 see also Grillner 1969 Budakova 1973 Forssberg & Grillner 1973)

e) Modifiability of placing reactions during different conditions

If an animal standing on all four limbs is mechanically stimulated on the foot dorsum postural adjustments occur first in which the contra and the homolateral limbs increase the sup

port while the stimulated and the diagonal limbs decrease their supporting force There is a small early activation of the flex ors of the stimulated limb but only after the postural adjust ments (mean 480 ms) a real flexion and a subsequent placing occur (Massion & Smith 1973 1974) The same kind of stimulus evokes in a cat with free pendant limbs instead fast ipsi and contralateral flexions (80 200 ms) (see above) A short and weak electrical pulse (1 mA 1 ms) applied to the foot dorsum elicits a flexor burst with a latency (25 ms) corresponding to that during tactile placing If the animal stands however this reflex burst is depressed and instead a faster burst with shorter la tency emerges (paper V Fig 9) These findings imply that there are several alternative programs that may be activated by the same tactile stimulus Different short latency pathways may be selected and the generation of flexion may even be delayed until appropriate postural adjustments have been made and the contra lateral limb may be extended or flexed The program utilized is influenced by the ongoing motor activity In the next chapter it will be shown that the locomotor activity to a large extent can control the reflex pathways from the foot dorsum

a) The reaction

Cats are able to avoid most obstacles during forward locomotion but they need to deal with unexpected pertubations If an obstacle impedes the forward movement of the foot during locomotion a short latency reflex activation of the flexor muscles occurs and as a result the limb is briskly flexed and lifted above the obstacle (see Fig 4) During early swing an enhanced

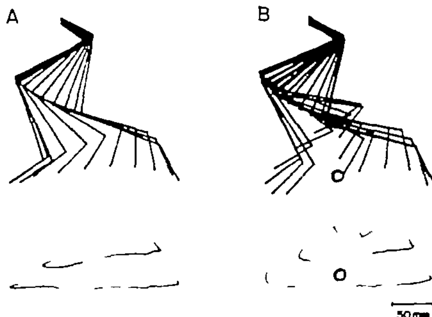


Fig 4 Movement analysis in an intact cat walking on the tread mill of A: a normal swing phas and of B: a stumble corrective reaction licited by tick inserted in front of the limb during the swing phase The movements are ecorded by a S l pot System from which the position of the hip p lvis (iliac crest) kn e ankl and tarsometata s l j ints ar btained (ee Methods in paper V) At the top the movement of the l ft limb i displayed with stick diagrams in which traight line between the j ints (se above) represent consequtiv limb positions at 30 ms intervals (limb moves from right to l ft) Below the sti k diagrams the trajectori of the paw (t rsometatarsal joi t) and ankl are plotted with 6 ms int rval in lation t the p l i The pelvi is fixed in the ho izontal di ection in both ti k diagrams and traj to ı

flexion is evoked but later when the knee and ankle have started to extend a new flexion begins On the other hand when the dorsum of the foot is similarly stimulated during the support phase no flexor responses appear but instead a reflex burst preceded by inhibition is evoked in the extensors and a very brisk flexion is induced in the following swing phase In the spinal cat the extensor excitation is strong enough to induce an enhanced extension which pushes the animal further forward upward The reflex responses are thus phasically modified to produce reactions adapted to compensate for disturbances in accordance with instantaneous and dynamically changing conditions of the limb in different phases of the stepcycle It has been called the stumbling corrective reaction (papers IV and V)

b) Receptors and receptive fields

If the skin is anaesthetized before contact the foot instead of being briskly lifted over the obstacle continues to push against it and is then pulled over the obstacle (Forssberg et al 1975 Prochazka 1978 paper IV and V) Thus skin receptors (touch) are one main source for the reaction Proprioceptors can, however induce activity that may stabilize the joints during the reaction (see Prochazka 1978) Different types of stimulation of the foot dorsum consisting of an airpuff (hair receptors) a mechanical contact (including mechano receptors) a weak (1 mA 1 ms) or an intense electrical pulse (10 mA 10 ms including nociceptors) all evoke a short latency flexor response during the swing phase depressed during the support phase and similar stumbling corrective reactions (paper V)

Cutaneous receptors located more proximal on the limb can elicit a reaction in which the limb is lifted higher than after foot contact Even a stimulus to the hairs on the abdomen evokes *a similar reaction This is meaningful as a high obstacle that* impedes locomotion will first touch the belly leading to an en hanced flexion even before the limb has hit the obstacle (paper V)

c) Reflex pathways

Five different reflex pathways involved in the reaction have been identified by an electrical stimulus (1 mA; 1 ms)

applied to the dorsum of the foot during treadmill locomotion (paper V) There is one early reflex in the flexor muscle (St) with a latency of about 10 ms and one later occurring about 25 ms after the stimulus Two corresponding bursts can be found in the extensor muscles one at about 10 ms (LG) and a second with a longer latency (25 50 ms) In addition an inhibition starts around 10 ms after stimulation with a duration varying in different extensor muscles until 25 50 ms after stimulation The same kind of tactile stimulus can evoke independent but not simultaneous activity in all 5 pathways (see phasic gating below)

The early flexor excitation and the extensor inhibition correspond to the flexor reflex effects (Sherrington 1910 see Creed et al 1932) This reflex can be elicited by all types of afferents except group I fibres (Eccles & Lundberg 1959a b) It is possible that the corresponding reflex responses of the stumble corrective reaction are elicited by the same pathways Also in the flexor reflex the flexion in different joints could be modified depending on the site of the stimulus i e the local sign (Creed et al 1932) Earlier Sherrington noticed that a stimulus that evoked the flexor reflex could elicit a flexor inhibition or an extensor excitation under other conditions (Sherrington & Sowton 1911a b Cooper et al 1927 see also Holmqvist & Lundberg 1961) Intracellular recordings from triceps surae motoneurones revealed that a cutaneous stimulation usually evoked a combined EPSP IPSP of which excitation dominated in fast motor units and inhibition in slow ones (Burke et al 1970) The EPSP corresponds to the early extensor excitation of the stumble corrective reaction during the swing phase and the IPSP to the inhibition during the support phase No flexor inhibition could be evoked by paw stimulation during walking in any preparation as recorded by EMG

The latencies of the later bursts are consistent with a transmission to supraspinal centers and back (Shimamura & Livingston 1963) The responses occurred however in spinal animals and can thus be induced by spinal networks (see paper V) Hagbarth (1952) described how cutaneous stimuli could evoke specific excitatory and inhibitory effects depending on the stimulus site in spinal cats with latencies corresponding to both the earlier and the later reflex bursts of the stumble corrective

reaction The extensor muscles were inhibited from most parts of the limb but-excited from a skin area localized mainly over the muscle itself The flexor muscles were usually excited except from the area above the antagonistic extensor muscle

The stumble corrective reaction could be elicited from different types of receptors on the dorsum of the paw with only minor differences of the reactions Hagbarth (1952) showed that the responses after skin stimulation was the same either small or large fibres were activated In both studies however as in the flexor reflex (local sign) the responses depended on the stimulus site

It is tempting to relate the late flexor response of the stumbling corrective reaction to the tactile placing as the same type of stimuli evokes both reactions and the latency of the tactile placing is the same as the late flexor burst i e 25-30 ms (cf Amassian et al 1972 Norrsell & Lundberg in Lundberg 1973). It was previously suggested that tactile placing consists of a step generated by the locomotor generator and a directional specific reflex that induces the step and directs it in the lateral or medial direction (see above) The initial flexor activity (25 30 ms) can then either be the result of a reflex activity or constitute the beginning of the flexor locomotor burst The fact that the latency is similar during ongoing locomotion may suggest that there is first a reflex activation of the flexors which later can be maintained by the locomotor generator which is initiated by the reflex The response evoked in the stumble corrective reaction would then reflect the reflex part of the tactile placing superimposed on the already ongoing locomotion with a specificity depending mainly on the stimulus site

The intensity and the duration of the stimulus can however also influence the response A weak and short stimulus evoked much smaller reactions than a longer During the support phase a weak but long (1 mA train 40 ms) or an intense and short (10 mA 10 ms) electrical stimulus on the foot dorsum evoked only the characteristic extensor responses In contrast an intense and long (10 mA; train 40 ms) stimulus evoked a concomitant flexor response (i e a flexor reflex breaked through)
V) The transmission in the reflex pathways to the fl
thus be inhibited to an extent that prevents effects

trains in large afferents or short pulses in smaller ones (noci ceptive) A long spike train including small nociceptive afferents will however elicit a flexor reflex The difference can either be due to a summation of the compound signals from the different afferents or to a more specific effect from the small nociceptive fibres with a strong projection to the flexors This latter response clearly does not belong to the stumbling corrective reaction (paper V)

d) Differences of responses in intact and chronic spinal cats

The clearest difference of the stumbling corrective reaction between intact and spinal cats was the amplitude of the reflex responses During spinal locomotion a small extensor inhibition and a large excitation resulted in an enhanced extension during the support while an increased inhibition and a supressed exci tation canceled each other in the intact cat There were smaller differences in the early excitation of both extensor and flexor muscles while the late flexor response was larger in the intact preparation (see paper V Table I)

Many descending pathways such as cortico- rubro- and different reticulospinal pathways are known to influence the transmission of spinal cutaneous reflexes (Holmqvist & Lundberg 1961 Lundberg & Voorhoeve 1962 Engberg 1964 Engberg et al 1968 Hongo et al 1969 Burke et al 1973 Grillner & Shik 1973) Some of these pathways are phasically active during locomotion (Orlovsky 1970 1972) and it may be expected that the degree of excitability in the reflex arcs can vary between the intact and spinal conditions The difference of the reflex bursts can be due both to phasic and tonic changes of the transmission in the relevant spinal pathways and also to complementary supra spinal loops (see Fig 3)

e) Phasic gating

The stumble corrective reaction is phasically adapted to produce functionally meaningful reactions in each phase of the stepcycle The phasic modulation of the four excitatory reflex responses is shown in Fig 5 in which the amplitude of the rectified and filtered EMG-responses is plotted in the different phases of the stepcycle in an intact walking cat The early and

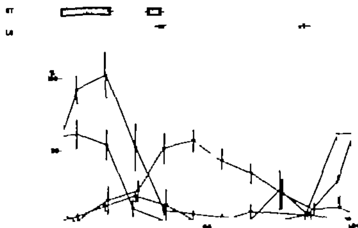


Fig 5 Phas dependenc f the reflex respons in St and LG during treadmill l comotion in an intact cat The paw i lectrically timulated (2 mA, 1 ms) The mean amplitud f rectified and filt ed EMG responses (±SE) is alculated f each 10th f the stepcycl expressed as a pro entage of the mean maximal f 10 normal locomot r bursts and pl tted versus the phase f timulation in the tepcy l The tepcycles are normalized to the onset f the main activity in St The schemati EMGs present 10 normal locomot burst (±SD) The symbol f ing St and LG ar used in the gr ph in whi h open symbol indicat the early flex responses (ca 10 ms) and the filled the later response (25–30 ms)

the late flexor responses but also the early extensor response (though small) are largest during the flexor period while the late extensor response is largest during the extensor period (see also Fig 6A) The fifth reflex response i e the extensor inhibition is present when the extensor muscles are active Whether an inhibition occurs also during other periods cannot be investigated by EMG-recordings

The relative amplitude of the responses is phasically modulated in a qualitatively similar way in walking spinal cats (see Fig 6B and above) which implies that a spinal mechanism related to locomotion would act as a gain control The simplest explanation would be that all reflex pathways are continuously open and that the phasic changes of the motoneuronal membrane potential that occurs in each stepcycle (Edgerton et al 1976) would determine whether or not the synaptic potentials will be sufficient to reach the threshold to discharge the motoneurones Such a mechanism cannot be responsible for all the effects as e g the early extensor burst which is largest during flexor

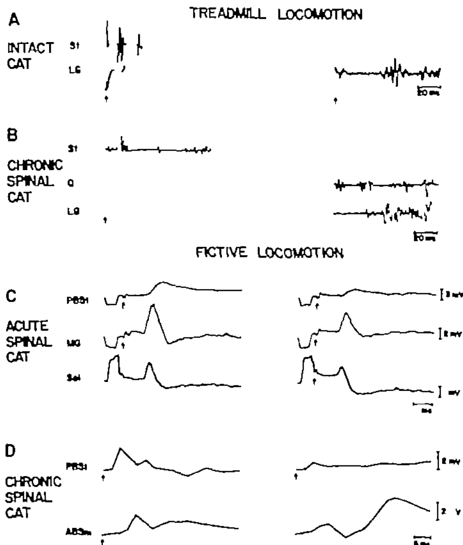


Fig 6 Reflex responses evoked by an electrical pule (1 mA 0 5 ms 5 mA 5 ms) during diff ent period of locomoto activity in diff rent p eparations The flexo p iod is defined as the interval from the one t f flexor activity (St) t the one t of extensor activity and the extensor p riod as occupying the remaining part f the locomot cycl In A and B singl reflex esponses are recorded in the muscl by EMG In C and D the responses re intracell l rily recorded from the motoneu ones In C the responses re averaged on an HP 21 MX comput r (n 12 28) and in D calculated from scill gr ph recordings (n=18-37) The stimulus is indicated by an rrow In C a onductance puls (constant current) is injected through the microelectrod prior t the stimulus

activity (see Fig A C D) or the two reflex responses to the knee flexor (St) which both are large during the first locomotor burst but depressed during the second smaller one (see Fig 5) A more complex mechanism would be a phasically changed efficiency of the transmission in the reflex pathways at a premotoneuronal level Such a phasic gain control can be exerted either (i) by the spinal locomotor generator that would influence the elements of the reflex pathways concomitant to its efferent outflow to the motoneurones or *(ii)* by interaction from peripheral afferents that are phasically activated by the locomotor movements (see Fig 10 in paper IV)

Curarized low spinal cats performing fictive locomotion (Grillner & Zangger 1974 1975 1979) were used to explore the neural mechanism of this gating In these preparations rhythmic activity related to locomotion is elicited by means of noradrenergic precursors or receptor stimulators The neuronal activity can be recorded in peripheral nerve filaments and of course with micro electrode techniques One advantage for our purpose is that the central locomotor generator is active while the movements are blocked Electrical stimuli applied to the dorsum of the paw which in the walking cats evoked stumble corrective reactions evoked in the motoneurones several combinations of excitatory (EPSPs) and inhibitory postsynaptic potentials (IPSPs) Usually there was an EPSP in flexor motoneurones 7-8 ms after the stimulus and a corresponding EPSP followed by an IPSP in extensor motoneurones (see Fig 6C see also Burke et al 1970) Twenty two flexor and 37 extensor motoneurones from 12 cats were recorded (paper VI and unpubl) The amplitude of the EPSPs of both flexor and extensor motoneurones was usually largest during flexor activity and they were significantly larger in 9 flexor and 16 extensor motoneurones ($P<0.05$; Students t test see Fig 6C Fig 7 and also Edgerton et al 1976 Andersson et al 1977) The amplitude of the IPSP in the extensor motoneurones was often larger during extensor activity although it varied more (see Fig 6C Sol) Only occassionally late extensor EPSPs were evoked by paw stimulation A late reflex EPSP could be evoked in some flexor motoneurones but only during the early period of flexor locomoto activity (see Fig 1 3 in paper VI)

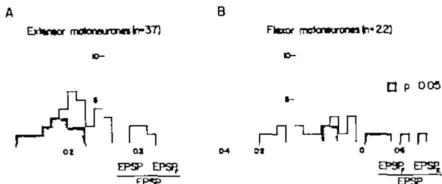


Fig 7 The relative modulation of the ea ly EPSP in acut ly pinalized cats pe f rming fi tiv l comotion The diff enc f the av raged amplitud (Fig 6C) b tween th flexor and extensor period (ee Fig 6) is normalized and plotted as histogram f extenso nd fl xor motoneurones (See F rmula in the figur) Shadowed units indicat motoneu on in whi h the r ponses re significantly (p<0 05) diff ent in the two period (Student t-test)

Intracellular recordings from a chronic spinal cat perfor ming fictive locomotion showed however also a second large EPSP with a latency of 20-40 ms in the extensor motoneurones This EPSP was much larger during the extensor activity (p<0 001; Students t test) while the early extensor and the flexor EPSP were larger (p< 0 01) during flexor activity (see Fig 6D) Late EPSPs were not seen in flexor motoneurones of the chronic spinal cat

Theoretically the observed changes in PSP amplitude could be attributed to pronounced changes in motoneuronal conductance due to the oscillation of the membrane potential (see Nelson & Frank 1967 Edgerton et al 1976) The finding of two EPSPs in the same motoneurone modulated independently of each other eliminate however this possibility (see Fig 6D) Furthermore the amplitude of conductance pulses (constant current) injected through the microelectrode prior to the stimulus did not change significantly during the cycle (see Fig 6C) The extracellular field potentials just outside the impaled cells were also routi

nely recorded (see paper VI)

The phasic modulation of the different EPSPs during fictive locomotion is in accordance with the modulation of the corresponding reflex responses during walking Since no phasic inflow from the periphery could be present there is clear evidence that the locomotor generator exerts a phasic gating between the different reflex pathways The most likely explanation would be an interaction on the interneuronal level although there could also be a presynaptic inhibition of alternative sets of peripheral afferents or gating at the dendritic tree of the motoneurones Two different possibilities may be suggested First there can be a common last order interneurone on which the efferent signals from the locomotor generator and signals from the reflex pathways converge This would imply that the signals would mutually facilitate each other before reaching the motoneurones Such a mechanism can however not explain the modulation of the early extensor EPSP which is largest during the flexor activity Another possibility would be that the spinal locomotor generator exerts a more selective phasic control with inhibition and/or facilitation of the transmission in specific interneuronal pathways (see Fig 8) The modulation of the reflex responses is thus at least in part a result of a phasic gating between alternative pathways exerted by the spinal locomotor generator The excitability level of the motoneurones is inevitably however of major importance for the final outcome of the responses and has thus in itself an effective gating function The amplitude of the early EPSPs were seldomely modulated more than 40% (see Fig 7) The low excitability level of the motoneurones reduces certainly the early reflex response of the extensor muscles although the pathway is facilitated during this period by the locomotor generator

A similar type of phasic gating was demonstrated in the swimming spinal dogfish in which reflex responses were switched between the two sides of the body dependent on the alternating locomotor activity (Grillner et al 1977 Wallén 1977) Wallén (1979; submitted for publication) showed that undulating movements of the body stabilized the rhythm (see also Grillner & Wallén 1977) bud did not influence the reflex responses

No peripheral feedback was needed for the phasic gating

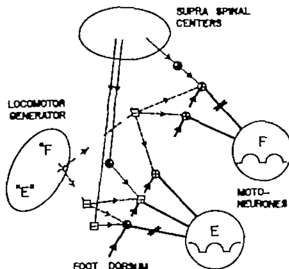


Fig 8 Hypothetical mod l of the phasic gain ontrol exerted by the spinal locomoto generat r and of the upraspinal control on the five reflex pathways from the dorsum f the foot to the flexor and extensor motoneurones The pathway ar d awn as thick lin Plus ( ) and minus (-) repr sent excitatory and inhibitory pathways respectively and th peripendi ula lines mark the long l tency pathways The influenc from supraspinal ent rs and from the locomoto gen tor on the individual pathways pl tted s dotted lin (plus and minus as abov) The influenc from the locomotor gen r t changes in phas with the ff ent output to the flexo F and extensor E motoneurones

in the cat (see above) It is however not known whether the phasic inflow during locomotion still assists the centrally exerted gain control or whether it as in the dogfish exerts no or little influence on the actual reflex pathways

During locomotion in thalamic cats a stimulus of the plantar region of the foot prolonged flexor and extensor activity in the respective phase of the stepcycle (Duysens & Pearson 1976) A stimulus of the dorsum of the hindfoot evoked during similar conditions an increased flexor or extensor activity in the ipsilateral forefoot depending on the phase of the stepcycle (Miller et al 1977) It was also found that PSPs in flexor and extensor motoneurones evoked from cutaneous sources other than the foot dorsum were phasically modulated during fictive locomotion (Schomburg et al 1977 1978 paper VI) That means that also other reflex pathways are controlled by the locomotor generator

Phasic gating is not unique for the locomotor system Brief

chemical stimulation of the carotid body or electrical stimulation of the carotid sinus nerve produced an increased inspiration and an increased discharge in the phrenic nerve during the inspiratory phase of the respiration (Black & Torrance 1967 Band et al 1970 Eldridge 1972a b) but increased the activity of expiratory muscles during expiration (Eldridge 1976) By extracellular recordings from single medullary respiratory neurones which fired during inspiration it was demonstrated that the signals from the chemoreceptors were phasically modulated before projecting to these cells (Lipsky et al 1977) Stimulation of the glossopharyngeal and superior laryngeal nerves evoked short latency excitation of the contralateral phrenic nerve during inspiration but not during expiration (Berger & Mitchell 1976) These responses were also slightly modulated prior to the inspiratory neurones (see above) but the excitability of the motoneurones (phrenic) was suggested to have a more significant function in the phasic gating (Berger 1978)

There is thus in principle the same type of phasic gating in different innate motor behaviours It is reasonable that gating not is restricted only to these systems but may generally occur concomitant to the efferent output in innate as well as learned central motor programs

GENERAL DISCUSSION

The control of movement is one very important function of the central nervous system The temporal and spatial activation of the motor units that execute a movement is the final result of this control At the same time as the nervous system controls all motoneurones involved in an ongoing movement it must transmit and solve a number of other control problems The more evolutionary advanced an animal the more complex and the greater the number of mental processes involved In order for higher levels of the nervous system to solve more intricate problems concomitant with a proper activation of each motor unit Bernstein (1967) suggested a hierarchical organization of the central nervous system where in the number of parameters controlled by the brain during a movement was reduced Several autonomic synergisms should be organized at a lower level of the nervous system ready to execute classes of stereotyped movements when called upon from higher centers in the hierarchy (see also Gelfand et al 1971)

The present findings are in agreement with such an organization and extend it, since rather complete integrative motor functions can be performed at a low level in the central nervous system (spinal cord) With the specific locomotor activity generated by the spinal cord a simple and unspecific control from higher levels would suffice to drive the networks faster or slower (see Grillner 1975 1979b Shik & Orlovsky 1976) The higher levels would in addition to regulating speed only exert a control of the direction of the locomotion the adaptation of the movements to the environment and the equilibrium

SUMMARY AND CONCLUSIONS

Spinal integrative motor functions involved in the neural control of locomotion have been investigated mainly by analysis of muscular activity and movements in chronic spinal cats

I In the spinal cord with intact reflex interaction neural networks can generate locomotion that resembles that of intact animals even in many details The locomotor generators that produce the basic rhythm are influenced from the periphery to provide proper speed adjustments of the locomotor activity The two limbs are properly coordinated by spinal mechanisms

II A basic reflex network that induces tactile placing is organized in the spinal cord The spinal networks are controlled from supra spinal centers

III A stumble corrective reaction is described which is phasically adapted to compensate for unpredicted disturbances of the limb in accordance with the instantaneous and dynamically changing conditions in different phases of the step-cycle Activity in several spinal reflex pathways contribute to the reaction The spinal locomotor generator is shown to phasically control individual pathways

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Variated Serum Gastrin Concentration
Trophic Effects on the Gastrointestinal Tract of the Rat

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G. LUNDQVIST, F. SUNDLER and J. THORELL

LUND 1979

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SUPPLEMENTUM 475

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Variated Serum Gastrin Concentration Trophic Effects on the Gastrointestinal Tract of the Rat

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Abstract

OSCARSON J., HÅKANSON R LIEDBERG G LUNDQVIST G SUNDLER F and THORELL J *Variated serum gastrin concentration. Trophic effects on the gastrointestinal tract of the rat*
Acta physiol scand 1979 Suppl 475

Gastrin is thought to be a trophic agent for the digestive tract and the pancreas This concept was studied on rats subjected to various operations designed to create hyper- or hypo-gastrinemia Elevated serum gastrin concentration resulted from vagal denervation antrum exclusion and fundectomy Reduced serum gastrin concentration resulted from antrectomy Antrum exclusion but not vagotomy increased the weight and height of the oxyntic mucosa; antrectomy had the opposite effects There were no trophic changes in the small and large bowel or pancreas attributable to the serum gastrin concentration In the bypassed duodenum (following antrum exclusion or antrectomy B II) the villus height was greatly lowered suggesting that in this location the passage of food acts as a trophic stimulant Our results show that endogenous gastrin exerts a trophic effect on the oxyntic mucosa; there was no evidence for a similar effect on the extragastric gut and pancreas

Key-words gastric surgery hypergastrinemia hypogastrinemia trophic action digestive tract

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INTRODUCTION

Gastrin is a physiological stimulant of gastric HCl Among other actions of exogenous gastrin and its analogues are trophic effects on the mucosa of the digestive tract except for the esophagus and gastric antrum (Johnson et al 1975 Barrowman 1975 Johnson 1976 Mak and Chang 1976) A trophic effect of gastrin on the pancreas has also been advocated (Barrowman 1975, Johnson 1976 Mayston and Barrowman 1973 Mayston et al 1975 Reber et al 1977) It has been argued that the trophic actions of gastrin are to be regarded as physiological (Johnson 1976 1977) Other gastrointestinal hormones have also been claimed to affect tissue growth and cell renewal (Johnson and Guthrie 1976) Exogenous cholecystokinin produces hypertrophy and hyperplasia of the pancreas (Petersen et al 1978) Secretin (Johnson and Grossman 1969) and somatostatin (Bloom et al 1974 Gomez-Pan et al 1975 Barros D Sa et al 1975, Arnold et al 1975 Konturek et al 1976 Vatn et al 1977 Phillip et al 1977) inhibit the secretagogue effects of gastrin Secretin (Stanley et al 1972 Wiseman and Johnson 1976) and possibly somatostatin as well counteracts the trophic action of gastrin It is not yeat clear which of these trophic or antitrophic effects should be considered physiological; so far endogenous gastrin has not been shown to have all the effects ascribed to exogenous gastrin Thus in patients with gastrinoma hyperplasia was observed in oxyntic (Neuburger et al 1972) and duodenal mucosa but none was noted in ileal mucosa (Shimoda et al 1968 Hansbach et al 1968) Variations in the serum gastrin concentration affected neither the starvation atrophy of the small bowel nor the hyperplastic response after jejunectomy (Oscarson et al 1977) No effect was noted in small and large bowel mucosa after longstanding changes in the serum gastrin concentration (Oscarson et al 1979)

Treatments that raise or lower the serum gastrin concentra-

tion have been found to affect the oxyntic gland area of the stomach in various ways A correlation between endogenous serum gastrin levels and rate of synthesis of DNA has been reported (Willems et al 1977 Casteleyn et al 1977) Upon transposition or exclusion of the antrum the height of the oxyntic mucosa was increased as was also the number of parietal cells chief cells (Lehy et al 1973) and endocrine cells (Alumets et al 1979) Antrectomy which results in hypogastrinemia reduced the size (Martin et al 1970) and the height of the oxyntic mucosa (Lehy et al 1973) the size and volume density of parietal cells (Capoferro and Nygaard 1973 Helander 1976) and the number of endocrine cells (Håkanson et al 1976)

Available information suggests that endogenous gastrin exerts trophic control of the oxyntic mucosa possibly also of the duodenum while its importance for the small and large bowel and for the pancreas is doubtful In the present communication we describe the effects on gut and pancreas of various gastric operations resulting in widely differing serum gastrin concentrations

MATERIAL AND METHODS

Male Sprague-Dawley rats weighing 200-250 g purchased from the same breeder were used The rats were housed in plastic cages and given commercial rat food pellets and water The animal quarters were lighted in alternate cycles The rats were randomly allocated for the experimental groups Control rats were unoperated Vagotomy was abdominal and the added pyloroplasty was in the Heinecke-Mikulicz fashion One group of rats had pyloroplasty only Antrectomy was carried out by removal of the distal half of the glandular stomach including the entire lesser curvature followed either by gastroduodenostomy end-to-end (Billroth I) or gastrojejunostomy end-to-side (Billroth II) Antrum exclusion was performed by transecting the stomach between the oxyntic and antral mucosa

closing the antrum and reestablishing the gastrointestinal continuity by a gastrojejunostomy end-to-side The vagal innervation was spared Fundectomy involved resection of the entire oxyntic gland area followed by plication of the rumen and anastomosis of the antrum to it; vascular and vagal trunks were preserved (Fig 1)

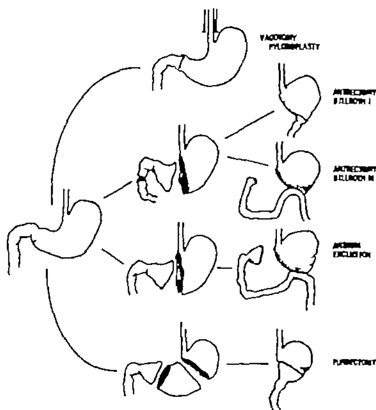


Fig 1 Schematic p es ntation of vag l denervation and the v ious ga t io r ections

Food was withdrawn the evening before operation and feeding was resumed the following day Operations were performed under diethyl ether anesthesia No antibiotics were used Survival rate varied from 53 to 100% (Appendix Table I) Postoperative weight gain was similar in all groups with the exception of vagotomy (see also Mordes et al 1977) (Appendix: Fig 1) Ten weeks after the operation half the number

of rats in each group were fasted for 48 hours in individual cages with wire mesh bottoms the others were freely fed The animals were killed between 10 a m and 12 noon by exsanguination from the abdominal aorta under light diethyl ether anesthesia Serum and plasma (taken in heparinized tubes containing 400 KIE Trasylol^R per ml blood) was stored at -30°C The pancreas was dissected out in toto frozen on dry ice and weighed The stomach was removed opened along the major curvature rinsed with iced 0 9% NaCl solution and weighed The opened stomach was flattened on a wooden board with the mucosal surface upwards and a standard punch (5 mm in diameter) was taken with a punch press from a predetermined site on the oxyntic gland area (Fig 2)

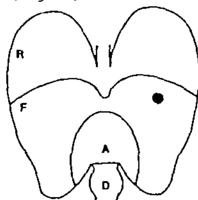


Fig 2 Schematic presentation of the site and size of the fundic punch R rumen; F fundus (oxyntic gland area); A antrum; D duodenum

The punch was weighed and frozen in liquid propane-propylene for histological examination (see below) The oxyntic and antral mucosa were scraped separately off the gastric wall with a scalpel frozen on dry ice and weighed From the duodenum two specimens (each one cm long) were taken one immediately below the duodenal bulb (proximal duodenum) and one 5 cm more distally (distal duodenum) They were rinsed in iced 0 9% NaCl solution gently blotted weighed and frozen for histology in liquid propane-propylene

The small and large bowel were removed and specimens (5 cm

in length) were excised from the mid small and mid large bowel gently flushed with iced 0 9% NaCl solution opened and the mucosa was scraped off the muscular wall with glass slides The mucosal scrapings were frozen on dry ice and weighed Material for chemical analysis was stored at $-30^{O}C$

Mucosal scrapings and pancreas were thawed and homogenized in 5% (w/v) citric acid in 0 25 M sucrose RNA and DNA were measured by the technique of Scott et al (1956) as modified by Hinrichs et al (1964) Aliquots of the homogenates were extracted with cold 0 3 N perchlorid acid (PCA) The combined supernatants were washed with cold 80% ethanol and with a mixture of equal parts of ethanol and diethyl ether; the supernatants obtained contained mostly lipids The residues were then digested in 1 N NaOH for 1 hour at room temperature Upon addition of 6 N HCl DNA and proteins precipitated leaving the RNA fraction in the supernatant The residues were washed with a 1:5 mixture of 6 N HCl and 1 N NaOH and this wash was added to the RNA fraction The residues were digested in 1 N PCA at $60^{O}C$ The digestion was repeated and the supernatants combined to constitute the DNA fraction The amount of RNA and DNA in tissue were determined by ultraviolet spectrophotometry

Protein in the crude tissue extracts was measured with the colorimetric method of Lowry et al (1951)

For histological examination the specimens frozen in propane-propylene were freeze-dried fixed in formaldehyde vapor (Björklund et al 1972) and embedded in paraffin Sections were cut perpendicular to the mucosal surface and stained with hematoxylin and eosin Examination was with objective x 6 3 and eyepiece x 8 (visual field diameter 2 5 mm) At least 10 determinations of oxyntic mucosal height and proximal and distal duodenal villus height and crypt depth were made on at least 2 sections from each animal

Gastrin in serum was measured using the radioimmunoassay described by Stadil and Rehfeld (1971 1973) Antibodies

against synthetic human gastrin I (residues 2-17) were supplied by Professor Jens Rehfeld Institute of Medical Biochemistry University of Aarhus Denmark Radioiodinated heptadecapeptide gastrin I was prepared by the technique of Stadil and Rehfeld (1972) Incubation was at $4^{o}C$ for at least 48 hours Free tracer was separated from bound by the addition of anion exchange resin as described by Rehfeld and Stadil (1973) Results were expressed as pg equiv synthetic human gastrin I per ml

Plasma somatostatin was determined by a solid phase radioimmunoassay (Arimura et al 1979) with somatostatin antibodies (a generous gift from Dr Robert Elde Department of Anatomy University of Minnesota Minneapolis USA) coupled to cyanogen bromide activated cellulose according to Wide (1969) The antiserum has been characterized earlier with respect to its crossreactivity against different parts of the somatostatin molecule (Arimura et al 1979) as well as to its lack of crossreactivity against other gastrointestinal peptides (Lundqvist et al 1979) Before radioimmunoassay plasma samples were extracted with aceton-petroleum ether as previously described (Arimura et al 1979) Results were expressed as pg equiv synthetic ovine somatostatin per ml

Statistical significance was assessed by analysis of variance Rats with no operation were used as control If heterogenity of group means between treatment group was observed the confidence interval at p = 0 99 for the control group was calculated Means outside this confidence interval were considered different from the control Vagotomy and pyloroplasty was compared to pyloroplasty alone

RESULTS

Gastrin and somatostatin in blood The diurnal variation in serum gastrin concentration of unoperated freely fed rats was followed in one experiment which showed about 3 times difference between zenith and nadir; zenith was at about 12

midnight and nadir at about 8 a m (Fig 3)

In the operated rats the highest serum gastrin concentration was observed after fundectomy (3 5 times higher than in freely fed unoperated animals) (Fig 4) while the lowest serum gastrin concentrations were recorded after antrectomy (10-20 per cent of that in freely fed unoperated animals) In the freely fed state the serum gastrin concentration was about the same in unoperated rats and following pyloroplasty vagotomy and antrum exclusion In unoperated rats fasting for 48 hours lowered the serum gastrin level to about 20% of that in the freely fed state Fasting did not lower the serum gastrin level after antrum exclusion or fundectomy After vagotomy fasting induced only a slight reduction ($p < 0$ 02)

Although the plasma somatostatin concentrations generally tended to be higher in fed than in fasted animals (Appendix: Table II) there was no correlation with the serum gastrin concentration There was a tendency for the various gastric resections to lower the plasma somatostatin concentration (in freely fed rats)

Organ weight and mucosal height The stomach was heavier after vagotomy than after pyloroplasty alone (Fig 5) This increase was not in the weight and height of the oxyntic mucosa (Fig 5 6) Also the antral mucosa was slightly heavier after vagotomy than after pyloroplasty alone (Fig 5) After antrum exclusion a pronounced increase was noted in the weight of the fundic punch (Fig 5) The oxyntic mucosa was higher in antrum excluded rats than in unoperated rats and lower after antrectomy (Fig 6)

Specimens from the bypassed duodenum (after antrectomy BII or after antrum exclusion) weighed less than specimens from the duodenum left in continuity (after antrectomy BI) (Fig 7) No other surgical procedure significantly afftected the weight of the duodenal wall Also the villus height of the duodenal mucosa was greatly reduced after antrum exclusion and antrectomy BII while after antrectomy BI no decrease

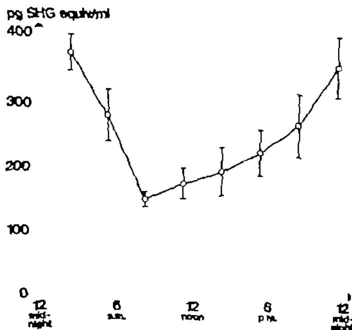


Fig 3 Diurnal variation of serum g trin concentration of freely fed rats n=5 for each time

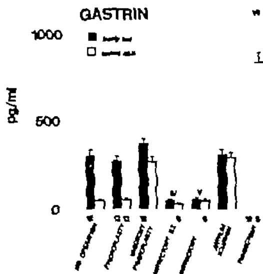


Fig 4 The serum gastrin concentration of the v rious experimental groups $p < 0\ 01$ for III v II (freely fed) $< 0\ 001$ v II (fasted) $p < 0\ 001$ for IV and V v I (freely fed) $p < 0\ 001$ for VI v I (fasted) $p < 0\ 001$ for VII v I

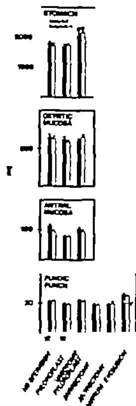


Fig 5 W ight of the whole tomach (a) oxyntic (b) and antral (c) mucosa and of the fundic pouch (d) Antrum exclusion and antrectomy are not included because of the erro introduced by the resection $p < 0\ 001$ for IIIa v IIa $p < 0\ 001$ for IIc v IIIc and Ic $p < 0\ 005$–$0\ 001$ fo VId v Id

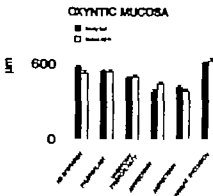


Fig 6 Height of the oxyntic mucosa in µm $p < 0\ 001$ for IV and V v, I $p < 0\ 01$ fo VI v I

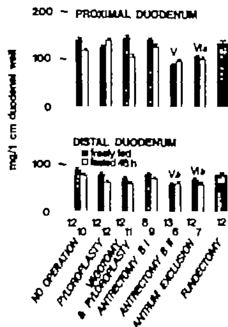


Fig 7 Weight in milligrams of 1 cm long specimens of the proximal (a) and distal (b) duodenal wall p < 0 005 0 001 for Va and VIa v Ia p < 0 005 0 001 for Vb and VIb v Ib

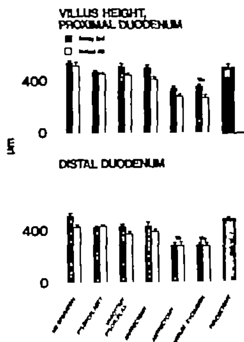


Fig 8 Villi s height in μm in proximal (a) and distal (b) duodenal mucosa fter the various operations p < 0 001 for Vab and VIab v I

was noted (Fig 8) Crypt depth was somewhat reduced following antrectomy BII but the effect was not statistically significant (Appendix: Table III)

The weight of small bowel mucosal scrapings (per unit length) of fasted and fed animals was unchanged after the various surgical procedures; the same applied to the weight of large bowel mucosal scrapings of fasted animals (Appendix: Table IV) In fed animals antrum exclusion but not fundectomy increased the weight of large bowel mucosal scrapings

The pancreas was heavier after antrum exclusion than after antrectomy (Fig 9)

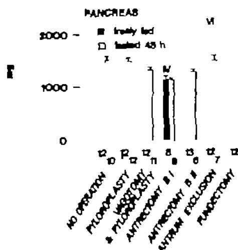


Fig 9 Weight of the pancreas after the various operations $p<0\ 005-0\ 001$ for IV v I $p<0\ 001$ fo VI v I

Nucleic acid and protein content Changes in the content of DNA and RNA in the oxyntic mucosa induced by hyper- or hypogastrinemia could not be followed due to partial loss of the organ as a result of gastric surgery

In the small bowel mucosa no changes in DNA RNA or protein content were detected after the various gastric operations (Appendix: Tables V-VII) In the large bowel mucosa there was a tendency for the DNA and protein content to

increase after vagotomy and antrectomy BI

In the pancreas the protein content was increased by bypassing the duodenum (antrum exclusion and antrectomy BII) but nucleic acids and protein were not consistently affected by all other operations; i e changes in fed and fasted groups were not parallel (Appendix: Tables V-VII) Thus no correlation with the serum gastrin concentration was noted

DISCUSSION

The purpose of the present study was to establish whether the trophic effects of exogenous gastrin could be simulated by treatments that produced high serum concentrations of endogenous gastrin The trophic effects of gastrin on oxyntic mucosa are widely accepted and there is much evidence that this is a physiologically important action (for recent reviews see Johnson 1976 1977) However the question remained: Would endogenous gastrin stimulate growth also of small and large bowel mucosa and of pancreas as claimed for exogenous gastrin? The various surgical interventions employed in the present study resulted in markedly different serum gastrin concentrations Fundectomy greatly raised the serum gastrin concentrations probably because of the high antral pH while antrectomy lowered the serum gastrin concentration Vagotomy and antrum exclusion were less effective in raising the serum gastrin concentration Although serum gastrin in the fasted state in these groups was elevated (probably as a result of high antral pH) this study gives no evidence that the serum gastrin levels in freely fed antrum excluded or vagotomized rats greatly exceeded those in control rats It was therefore not clear whether a trophic effect was to be expected at all However antrum exclusion and fundectomy produced a permanently raised serum gastrin concentration in contrast to the undulating serum gastrin concentration in freely fed unoperated rats Not surprisingly therefore antrum exclusion increased the weight of the fundic punch

$(p < 0.005)$ and the height of the oxyntic mucosa $(p < 0.01)$ Antrectomy on the other hand lowered the height of the oxyntic mucosa $(p < 0.001)$ Thus perturbation of the serum gastrin concentration is accompanied by changes in the oxyntic mucosa

Also vagotomy raised the serum gastrin concentration The absence of a trophic effect of vagotomy on oxyntic mucosa a finding in keeping with earlier reports (Helander 1976 Crean et al 1969 Ley et al 1973) may be explained by a diminished responsiveness to gastrin perhaps because of an antitrophic effect elicited concomitantly Alternatively the concentration of circulating gastrin was too marginally increased to produce a trophic effect

Not even with the marked hypergastrinemia following fundectomy was a trophic effect seen in the remainder of the digestive tract Stimuli other than gastrin were effective however Thus the weight and villus height of the bypassed duodenum was greatly reduced after both antrum exclusion and antrectomy BII despite the fact that antrum exclusion produced hypergastrinemia and antrectomy hypogastrinemia By contrast the duodenum was unaltered after antrectomy BI and fundectomy Both these operations allow food to pass through the duodenum Hence intraluminal factors seem to be of great importance for the maintenance of the duodenal mucosa as proposed by Altmann (1974) Some workers (Mayston et al 1974 Hitznegg et al 1975) have argued in opposition to Johnson (1976 1977) that exogenous gastrin is without trophic effects on extragastric gut mucosa Our results suggest that this lack of effect applies also to endogenous gastrin (for a conflicting report see Mac Gregor and Way (1976)

The weight of the pancreas was increased after antrum exclusion and lowered after antrectomy BI These weight changes were accompanied by parallel changes in the RNA and protein content of the pancreas This is in agreement with the findings by Reber et al (1977) on rats subjected to

antrocolic transposition However since fundectomy and antrectomy BII did not change the pancreatic weight the present results do not support the view that endogenous gastrin is important for regulating the size of the pancreas

The results of the present study are in full agreement with the concept that endogenous gastrin exerts a trophic effect on the oxyntic mucosa However the magnitude of this effect is probably not determined by the serum gastrin concentration alone (see Hansen et al 1976) The nervous activity of the gut wall and conceivably other intestinal hormones may modulate the response to gastrin; in the latter instance by additional trophic or antitrophic effects The plasma somatostatin concentration was followed in a search for evidence that this hormone might be implicated In the present study there was no evidence that somatostatin interfered with the trophic effects of gastrin

It can be concluded from the present data that endogenous gastrin exerts a trophic effect on the oxyntic mucosa; however there was no evidence for a similar effect of endogenous gastrin on the pancreas and extra-gastric gut

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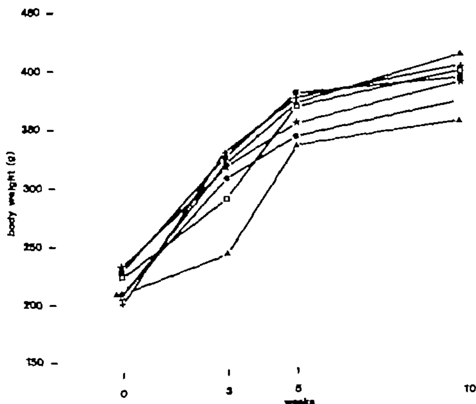
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APPENDIX

Variated serum concentration: Trophic effect on the gastro-intestinal tract of the rat

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ig 1 Weight development during the 10 postoper tive weeks Pyloroplasty △ vagotomy and pyloroplasty ▲ antrectomy BI □ antrectomy II ■ antrum exclusion ✱ fundectomy ● The group f with no oper tion ( ) i lso included

Table I Survival rates following the various operations

Surgical treatment	Survival/total number	Survival per cent
No operation	22/22	100
Pyloroplasty	24/24	100
Vagotomy and pyloroplasty	23/24	96
Antrectomy B I	17/32	53
Antrectomy B II	19/32	59
Antrum exclusion	19/33	58
Fundectomy	12/12	100

Table II Concentration of somatostatin in plasma

Surgical treatment	Freely fed	Fasted
	pg/ml mean ± S E (n)	
No operation	367 ± 35 3 (12)	146 ± 30 0††† (4)
Pyloroplasty	285 ± 38 9 (12)	190 ± 21 0† (6)
Vagotomy	261 + 35 5 (10)	228 ± 17 1 (6)
Antrectomy B I	198^{xxx}± 4 6 (6)	134 ± 20 2†† (5)
Antrectomy B II	223^{xxx}± 21 6 (12)	223 ± 45 7 (3)
Antrum exclusion	305 ± 22 9 (12)	234 ± 22 1† (4)
Fundectomy	215^{xxx}- 15 9 (12)	-

Stati tical significance is expressed throughout by x or † for 0 01 < p < 0 05 xx o †† for 0 001 < p < 0 01 and xxx or ††† for p < 0 001 The symbol x denotes the difference between control r ts and operated rats (within one vertical row) The symbol † denotes the difference between freely fed and fasted rats (within one horizontal row) No operation is control for pyloropla ty ntrectomy B I and B II antrum exclusion and fundectomy Pyloroplasty is control for vagotomy

Table III Crypt depth of the proximal and distal duodenal mucosa after the various surgical procedures[a]

	Crypt depth proximal duodenum		Crypt depth distal duodenum	
	Freely fed	Fasted	Freely fed	Fasted
No operation	212 ± 8 (12)	210 ± 16 (7)	204 ± 8 (12)	174 ± 10 (8)
Pyloroplasty	202 ± 8 (12)	198 ± 6 (12)	182 ± 4 (11)	176 ± 8 (12)
Vagotomy and pyloroplasty	202 + 8 (12)	176 ± 6 (10)	184 ± 8 (12)	158 ± 8 (10)
Antrectomy B I	214 ± 8 (10)	200 ± 14 (9)	186 ± 8 (8)	168 ± 10 (8)
Antrectomy B II	182 ± 10 (13)	170 ± 6 (6)	180 ± 6 (13)	170 ± 8 (6)
Antrum exclusion	200 ± 8 (13)	178 ± 12 (8)	185 ± 10 (13)	164 ± 6 (7)
Fundectomy	220 ± 8 (12)	-	198 ± 7 (12)	-

[a] Expressed as μm mean ± S E (n)

Table IV Weight of the small and large bowel mucosa [a]

	Small bowel		Large bowel	
	Freely fed	Fasted	Freely fed	Fasted
No operation	193 ± 8 (12)	140 ± $6^{\dagger\dagger\dagger}$ (10)	236 ± 10	190 ± $16^{\dagger}$
Pyloroplasty	191 ± 9 (12)	165 ± $6^{\dagger}$ (12)	232 ± 6	258 ± 16
Vagotomy and pyloroplasty	211 ± 7 (12)	147 ± $5^{\dagger\dagger\dagger}$ (11)	204 ± 8	292 ± $24^{\dagger\dagger}$
Antrectomy B I	213 ± 14 (8)	152 ± $8^{\dagger\dagger}$ (9)	220 ± 20	303^{xx} ± $24^{\dagger}$
Antrectomy B II	196 ± 7 (13)	166 ± $8^{\dagger}$ (6)	261 ± 20	240 ± 18
Antrum exclusion	235^{x} ± 15 (12)	176 ± $11^{\dagger\dagger}$ (7)	328^{xx} ± 14	227 ± $27^{\dagger\dagger}$
Fundectomy	196 ± 10 (12)	-	267 ± 14	-

[a] Expressed as milligrams of mucosal scrapings from 5 cm long specimens

Tabl V DNA cont t i small and large bowel mucosa and pancreas

	Small bowel		Large bowel		Pancreas	
	Freely fed ( )	F ted ( )	Freely fed	Fasted	Freely fed	Fasted
No operation	1 7 0 1 (12)	1 1 0 1†††(10)	2 2 + 0 1	1 5 + 0 2††	26 2	18 + 2
Pyl roplasty	1 7 0 1 (12)	1 3 0 1† (12)	2 3 + 0 1	2 2xx 0 1	22 + 2	18 + 1
Vagotomy and pyl ropl ty	1 6 + 0 1 (12)	1 0 0 04†††(11)	1 6xx 0 1	2 0 0 2	25 + 2	25xx 2
Antrectomy B I	1 8 0 2 (8)	1 1 0 1†† (9)	1 6 + 0 2	3 4xx + 0 2†††	11xxx+ 2	22 ± 2††
Antrectomy B II	1 6 0 1 (13)	1 3 0 2 (6)	2 4 + 0 2	2 1 + 0 3	16xxx+ 1	21 2
Antrum xclusion	1 8 0 1 (12)	1 2 0 1†††(7)	2 5 ± 0 3	1 7 − 0 3	22 + 1	38xxx+ 3†††
Fundectomy	1 9 ± 0 1 (12)		2 6 0 3		18x 2	

* Expressed mg pe 5 cm mucos (small and large bowel) r pe pancreas

Tabl VI RNA content in small and l rge bowel and pancreas

	Small bowel						Large bowel				Pancreas			
	Freely f d ( )			Fasted ( )			Freely fed		F sted		Freely fed		Fasted	
No ope tion	2 0	0 2	(12)	1 2	0 2†	(10)	2 1	0 3	1 8	0 2	75	4	53	3†††
Pyl ropl ty	2 2	0 2	(12)	1 5	0 2†	(12)	2 1	0 2	2 3	0 3	59	4	57	4
Vagotomy and pyl ropl ty	1 6	0 2	(12)	1 1	0 1††	(11)	1 8	0 2	1 6	0 1	61	3	67	4
Antrectomy B I	2 2	0 4	(7)	1 3	0 2	(9)	1 8	0 2	2 9^{xx+}	0 3††	49^{xxx}	4	50	3
Antr ctomy B II	1 8	0 1	(13)	1 1	0 3†	(6)	2 8	0 3	1 9	0 2†	54^{xxx+}	3	57	4
Antrum xclusion	2 2	0 1	(12)	1 3	0 3†	(7)	2 8	0 3	2 5	0 3	77	3	108^{xxx+}	8††
Fundectomy	1 8	0 2	(12)				2 5	0 2			76	5		

Expressed as mg pe 5 cm mucosa (small and large bowel) or per pancreas

Tabl VII Prot in content in small and larg bowel and pancreas [a]

	Small bowel				Large bowel				Pancreas			
	Freely fed( )		Fasted (n)		Freely fed		Fasted		Freely fed		Fasted	
No ope tion	34	2 (12)	18	$3^{\dagger\dagger\dagger}$ (10)	38	3	30	+ 3	290	+ 19	293	13
Pyl ropla ty	30	2 (12)	22	$3^{\dagger}$ (12)	40	+ 4	28	3	299	± 6	338^{x}	± $9^{\dagger\dagger}$
Vagotomy and pyloroplasty	34	3 (12)	20	$2^{\dagger\dagger\dagger}$ (11)	28	3	48^{xxx}	$3^{\dagger\dagger\dagger}$	290	9	337^{x}	$9^{\dagger\dagger}$
Antrectomy B I	34	3 (8)	27	4 (9)	32	+ 4	57^{xxx}+	$4^{\dagger\dagger\dagger}$	225^{xx}	7	280	- $9^{\dagger\dagger\dagger}$
Antrectomy B II	34	3 (13)	27	5 (6)	42	+ 4	34	5	357^{xx} +	8	349^{xx} +	11
Antrum exclusion	38	2 (12)	35	6 (7)	36	5	40	+ 6	514^{xxx}	12	347^{xx} +	$10^{\dagger\dagger\dagger}$
Fundectomy	27	3 (12)			32	5			269	7		

Expre sed mg per 5 cm mucosa (small and large bowel) or per pancrea

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DYNAMIC AND STATIC COMPONENTS IN THE MYOGENIC CONTROL OF VASCULAR TONE IN CAT SKELETAL MUSCLE

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CONTENTS

This summary is based on the following publications:

I GRÄNDE P O & BORGSTRÖM P 1978 An electronic differential pressure flowmeter and a resistance meter for continuous measurement of vascular resistance Acta Physiol Scand 102 224 230

II GRÄNDE P O LUNDVALL J & MELLANDER S 1977 Evidence for a rate sensitive regulatory mechanism in myogenic microvascular control Acta Physiol Scand 99:432 447

III GRÄNDE P O & MELLANDER S 1978 Characteristics of static and dynamic regulatory mechanisms in myogenic microvascular control Acta Physiol Scand 102:231 245

IV GRÄNDE P -O BORGSTRÖM P & MELLANDER S On the nature of basal vascular tone in cat skeletal muscle and its dependence on transmural pressure stimuli Acta Physiol Scand In press

V GRÄNDE P -O & MELLANDER S 1979 Beta adrenergic inhibitory interference with myogenic vascular reactivity during experimental intervention Acta Physiol Scand 106 87 89

VI GRÄNDE P -O 1979 Influence of neural and humoral beta adrenoceptor stimulation on dynamic myogenic microvascular reactivity in cat skeletal muscle Acta Physiol Scand In press

In the text these papers are referred to by their Roman numerals

INTRODUCTION

The maintenance of cardiovascular homeostasis in normal life and in pathophysiological stress situations implies continuous adjustments of cardiac performance and vascular tone so as to satisfy the highly variable circulatory demands of the different tissues The regulation of the peripheral vascular functions is accomplished by adjustments of excitatory and inhibitory influences on vascular tone in the various circuits of the circulatory system Such alterations of smooth muscle tone are effected by regulatory mechanisms o i ginating from sites within and outside the tissue itself i e via the local and central vascular control systems Although details of integrated central control remain to be clarified several of the specific mediators the neuro transmitters and the circulating hormones have been identified unequivocally Knowledge about the final links of the local control systems is much less complete in particular with regard to the so-called myogenic control system The present investigations serve to elucidate in some detail the receptor-ef factor characteristics of myogenic control and its mode of operation in the vascular bed of skeletal muscle

Previous concepts of myogenic vascul r control

In 1902 Bayliss put forward his classical hypothesis that the intravascular blood pressure via distension caused a direct stimulating action on the vascu lar smooth muscle and enhanced contractility contributing to resting vascular tone and its regulation The hypothesis was based on a series of careful though not entirely conclusive experiments and on analogies to the responses to stretch of other types of smooth muscle Bayliss concluded that these respon ses were of myogenic origin and the term myogenic reactivity has lat r by convention been sed to denote the ability of the vascular smooth muscle to respond to the mechanical force exerted by the blood p essure Although the term myogenic is a relatively non-specific classification of this particular response (which in fact might have contributed to some confusion in this field of research) this terminology is so commonly used that it was adopted also in the present se ies of papers

The myogenic hypothesis of Bayliss was seriously questioned for several deca des starting with a critical study by An ep (1912) who claimed that Bayliss experimental results could be explained by other mechanisms in pa ticular by

local metabolic influence The opinion was then quite commonly maintained for considerable length of time that variation in tissue metabolite concentration was the principal means for local regulation of vascular tone An apparent obstacle for further progress in this field of research was the considerable difficulties encountered in designing specific tests of the myogenic hypothesis without simultaneous experimental interference with the local metabolic vascular control system; another problem is the apparent vulnerability of the myogenic control system to experimental intervention (see below)

The myogenic hypothesis was however revived by later hemodynamic studies Especially important was the work by Folkow in 1949, who quite convincingly could demonstrate active myogenic reactions to changes in blood pressure under circumstances of relatively minor metabolic influence This investigation led to a great many other more detailed hemodynamic whole organ and vital microscopy studies in various tissues which further supported the myogenic hypothesis (e g Folkow and Öberg 1961 Mellander Öberg and Odelram 1964 Johnson and Wayland 1967 Johnson 1968 Baez Laidlaw and Orkin 1974) and with time to the now quite generally accepted compromise view that metabolic as well as myogenic mechanisms are important links in local vascular control and synergistically responsible for autoregulation of blood flow (for ref see Johnson 1964 Mellander and Johansson 1968)

Myogenic mechanisms also seem to be involved in other autoregulatory phenomena It was thus demonstrated by Folkow and Öberg (1961) and especially by Järhult and Mellander (1974) that there is an effective myogenic autoregulation of capillary hydrostatic pressure in skeletal muscle over a wide range of arterial pressures from 30 up to 170 mm Hg, serving to maintain the normal transcapillary Starling fluid equilibrium during arterial hypo and hypertension Mellander Öberg and Odelram (1964) demonstrated that increased vascular transmural pressure as occurs in dependent vascular beds during hydrostatic load caused an especially marked myogenic constriction of the precapillary sphincters in skeletal muscle and skin tissues This study led to the concept of myogenic autoregulation of transcapillary filtration during increased transmural pressure which effectively could prevent the theoretically expected oedema formation These whole organ studies strongly pointed to the fact that myogenic reactions were especially vivid in the arterial microvessels This view was later confirmed more directly by vital microscopy observations in the microcirculation (e g Johnson 1968 Baez Laidlaw and Orkin 1974) which demonstrated that increased vascular transmural pressure as all

cited by raised venous pressure indeed caused the predicted myogenic diameter changes of the small precapillary vessels Johnson and Wayland (1967) also showed distinct dependence of capillary flow periodicity and myogenic vasomotion upon change of intravascular pressure

Although all these studies provided strong support for the myogenic hypothesis it has not been universally accepted An alternative interpretation of the ac tive constrictor response to raised vascular transmural pressure was put for ward by Gaskell and Burton (1953) and Haddy and Gilbert (1956) who attributed this response to a local nervous veno arteriolar reflex mechanism This expla nation however was questioned on the basis of histological examinations which gave no evidence for local nerve plexa in the vascular wall and further on the basis of preserved responses after adrenergic blockade (for ref see Fol kow 1962) Yet such a veno-arteriolar reflex might exist in some vessels as suggested by recent expts on the vascular bed of adipose tissue in the human (Henriksen 1976) and may here operate in synergism with the myogenic mechanism during a transmural pressure change

Some authors (e g Guyton 1964) have more or less rejected the myogenic hypothesis on the basis of a theoretical reasoning that myogenic control implies a positive feedback that could lead to extreme myogenic effector reactions and to serious instability in vascular regulation Further it has been stated that myogenic control could be self limiting insofar that a myogenic smooth muscle shortening in response to raised vascular distending pressure automati cally would abolish the primary receptor stimulus if the receptors were sensitive to change in length (Gaskell and Bu ton 1953)

Arguments agai st the above-mentioned objections have been derived from knowledge of vascular smooth muscle physiology from circulatory studies and from mathematical mod ls of myogenic control A tentative description of the myogenic hypothesis was thus put forward by Folkow (1964) which implied that vascu lar disten ion increases the frequency of rhythmic contractions initiated by pacemake s and propagated to adjacent smooth muscl cells A static elongation beyond control length of the myogenic sensor element during the period of elaxation of the smooth muscle would then occur between repetitive myogenic contractions the latter causing enhan ed resistance to flow Another explana tio was given by Johnson and Wayland (1967) who postulated that the myogenic sensor element was coupled in series with the contractile units of the vasula t re and that the myogenic reactions might be elated to changes of wall tension

It is obvious, that the exact events are difficult to predict from in vivo observations due to the complicating effects of Laplace and Poiseuille s laws and of wall/lumen variations on wall tension A recent mathematical model study of myogenic vascular control which took such factors into account (Borgström and Grände 1979) can aid in our understanding of those problems Such mathematical analysis provided more direct support for the opinion that myogenic reactions are triggered by and related to change of wall tension It further contradicted the objection that myogenic control via positive feedback necessarily would imply instability in vascular regulation and predicted instead that extreme myogenic effector reactions would not occur in response to changed vascular transmural pressure Possible extreme effects of positive feedback in myogenic control would further be limited simply by the restricted upper frequency range of the myogenic pacemakers (Johansson and Mellander 1975) and also by the dilator influence of metabolites accumulating as a result of flow restriction during myogenic constriction (Berne 1964)

The vast majority of studies in this field of research thus provide strong evidence for Bayliss hypothesis, and the myogenic mechanism is now quite widely appreciated as an important factor in the development of active responses to changes in blood pressure This in turn implies that such a myogenic mechanism acts by increasing or decreasing the normal basal vascular tone Basal vascular tone in this context refers to the marked contractile state which at normal pressure remains on the arterial side of most vascular beds after elimination of known nervous and humoral excitatory influences (e g Löfving and Mellander 1956 Folkow 1962)

Various explanations for the basal vascular tone have been proposed For instance it has been postulated by Johansson and Bohr (1966) that there is a plasma factor which is responsible for the tonic activity in the small blood vessels but the evidence is so far incomplete and the nature of the factor still unknown It has also been suggested that oxygen acting directly on the vascular smooth muscle causes the normal vascular contractile state (Ross Fairchild Weddy and Guyton 1962 Guyton et al 1964) but more likely oxygen is just supporting contraction at basal tone rather than initiating it (cf Duling 1972) There is much to indicate however that basal vascular tone is of intrinsic nature (Folkow 1962) and in some way closely related to the above described myogenic reactivity Basal tone might then result from spontaneous myogenic automaticity in the vascular smooth muscle, but it might also require some specific continuing stimulus for its development and maintenance

for instance initiated by the arterial blood pressure distension as apparent ly envisaged by Bayliss Basal tone might even be established by somewhat dif ferent mechanisms in the various sections of the vascular bed in view of the documented heterogeneity of vascular smooth muscle in different parts of the circulatory system (e g Bohr 1965 Vanhoutte 1978) It is apparent that these hypothetical explanations of basal myogenic tone require further clarification

Some recent studies have added new dimensions to the concept of myogenic vas cular reactivity by description of the electrophysiological events and of a seemingly important rate sensitivity in myogenic control

In the previous studies myogenic vascular reactivity was analysed almost ex- clusively in terms of the active responses which develop upon a given steady state increase or decrease in vascular transmural pressure Such steady-state myogenic effector reactions might at least a priori be considered to be the result of a static stimulus related to the amplitude of the transmural pres sure change Some indication for the presence of a rate-sensitive or dynamic component in myogenic vascular control was first obtained from studies of the mechanical vascul r response to stretch (Sparks 1964) and from experimental and theoretical analyses of amplitude and frequency characteristics of pres sure i duced changes of flow resistance in the renal and coronary circulations (Basar and Weiss 1969) and from observations of increased vascular tone upon shift from steady to normal pulsatile pressure perfusion in skeletal muscle (Mellander and Arvidsson 1974)

Direct evidence for an important dynamic component in the myogenic vascular response to passive change in length was more recently obtained from in vitro observations on the single-unit smooth muscle of the rat portal vein(Johansson and Mellander 1975) a study which also elucidated the underlying electrophy- siological mechanisms The vascular smooth muscle preparation in this investi gation was exposed to a given passive static stretch or shortening applied at various rates of length (L) change dL/dt over the range between 3 and +3 % of the muscle length/s Static stretch by 40 % of the muscle length was shown to evoke only moder te excitatory effects in terms of a 10 15 % increase in spike discharge above the resting control value and a corresponding increase in active force Dynamic stretch during the period of increasing muscle length was much more effective causing graded excitatory responses which at high dL/dt values were up to 15 20 times greater than those evoked by static stretch Passive shortening at different rates caused graded inhibitory respon

ses below the control level complete inhibition of electrical and mechanical activity being observed at the highest negative test value of dL/dt Further analysis suggest that the dynamic myogenic response was more closely correlated to the rate of change of passive tension ($d\sigma/dt$) than to dL/dt (Sigurdsson Johansson and Hellander 1977)

These in vitro studies which seem to be the first direct electrophysiological demonstrations of myogenic vascular reactivity provided direct support for the view previously tentatively discussed by Folkow (1964) that the myogenically active vascular smooth muscle can be considered to act as a mechanoreceptor-mechanoeffector unit in which distension via effects on a pacemaker mechanism causes facilitation of spike-discharge propagated to the neighbouring smooth muscle effector cells The net effect of such mechano-electrical coupling in response to receptor deformation is changes in the frequency of spike generation and attendant active changes in mechanical activity or vascular tone The in vitro studies also made it clear that myogenic reactivity in the portal vein is dependent not only on the static characteristics of the pacemaker stimulus but also and more distinctly on its dynamic characteristics

Aim of the present investigations

The studies summarized in this paper deal with the myogenic control of the vascular bed in cat skeletal muscle and in particular with the problem whether such regulation is characterized by rate-sensitivity i e by a dynamic component in addition to a static component as previously demonstrated for myogenic reactivity in the rat portal vein in vitro (Johansson and Hellander 1975) Both these components in myogenic control were shown to exist in the studied vascular bed (lower hind leg muscles) The papers will describe in some detail the localization of these components their receptor effector characteristics and their possible functional significance for myogenic control in general and for the initiation and maintenance of normal basal vascular tone An analysis of the interaction between myogenic and adrenergic control in the muscle circulation was also performed

The expts were designed so as to permit analysis of static and dynamic myogenic reactivity to transmural pressure stimuli under conditions of almost negligible metabolic influence This was accomplished by changing vascular transmural pressure in the muscle region via alteration of extravascular pressu e thereby avoiding the quite large passive flow changes inherent in previous approaches in which arterial or venous pressure was altered

A differential pressure flowmeter and a resistance meter were constructed (paper I) to permit continuous and accurate recordings of arterial blood flow and of total resistance as well as segmental resistances in proximal arterial vessels microvessels and large veins in the vascular bed of skeletal muscle

Evidence for the existence of a static and a rate sensitive dynamic component in myogenic vascular control was presented in paper II Dynamic reactivity was here shown to be localized to microvessels smaller than about 20 to 25 µm (i d) on the arterial side

Paper III describes in greater detail the stimulus effector characteristics and the possible functional significance of the static and the dynamic components in myogenic microvascular control in skeletal muscle

In paper IV an attempt was made to elucidate the nature of the basal tone in various segments of the muscle vasc lar bed by determining its dependence on normal arterial blood pressure via static mean pressure distension and dynamic pulse pressure distension as well as its responsiveness to strong inhibitory transmural pressure stimuli The analysis suggested that the nature of basal tone is different in large compared to small arterial vessels

It is a well known fact that myogenic reactivity can be severely depressed by experimental intervention Expts in paper V demonstrated that this unexplained phenomenon to a major extent could be attributed to a β adrenergic inhibitory effect on myogenic reactivity exerted by catecholamine release from the adrenals

The interaction between adrenergic and myogenic regulatory mechanisms was analysed more systematically in paper VI which revealed that activation within the physiological range of the sympatho-adrenal β adrenergic vascular control system can modify myogenic eactivity significantly and in a manner which seems to refine integrated vascular regulation

METHODOLOGICAL CONSIDERATIONS

Experimental preparation

The expts were performed on a total of 73 cats (b wt 2 8 - 5 0 kg) after anaesthesia with α-chloralose and urethane Body temperature was kept at 38 $\pm$0 5°C

All observations were made on the acutely denervated vascular bed of the lower leg muscles of the cat hind limb The surgical preparation was similar to that previously described by Kjellmer (1964) with the skinned muscle region sepa rated from the body except for its main vascular connections the popliteal artery and vein

After heparinization arterial blood flow was diverted from the femoral to the popliteal artery via a shunt circuit with a T-tube connection which permitted measurements of arterial inflow pressure (AP) and close arterial administra tion of drugs Venous outflow was shunted from the popliteal to the external jugular vein and venous outflow pressure (VP) was monitored from a T-tube close to the muscle region In the majority of expts regional blood flow was mea sured on the arterial side with a specially designed pressure gradient flow-meter (see next chapter) which was found to be superior to an electromagnetic flowmeter or a venous drop recorder unit used in some of the early expts The muscle region was autoperfused from the animal in all expts except for those described in paper VI and in one series of expts in paper IV where perfusion instead was accomplished with the aid of a pressure servo-controlled roller pump inserted in the arterial shunt circuit This pump could deliver normal pulsatile or non-pulsatile flows Arterial inflow pressure could be adjusted to desired levels with a screw clamp placed around the arterial shunt circuit (paper IV) An inert material (silicone rubber) was used for all tubes in contact with blood (shunts flowmeter perfusion pump) creating an entirely smooth inner surface of the tubings

To be able to follow not only total vascular resistance in the muscle region but also segmental resistances pressures were monitored from two additional sites along the vascular tree from artery to vein viz from arterial microves sels (small artery pressure SAP) and from venous microvessels (small vein pressure SVP) These pressures were measured via small catheters inserted in

the sural artery and vein according to a technique modified from that originally described by Haddy et al (1954) and later used in several studies (e g Johnson and Hanson 1962 Folkow Sonnenschein and Wright 1971 Lundvall and Järhult 1976) AP SAP SVP and VP were recorded with Statham transducers

Th muscle preparation with intact circulation was placed in a specially designed hermetically cl sed temperature-controlled (38°C) plethysmograph (Grän de Järhult and Mellander 1974) filled with Tyrode s solution The fluid in the plethysmograph was in connection via a tubing with an external open reservoir mobile in the vertical direction to permit variation of the hydrostatic fluid pressure in the plethysmograph and hence of extravascular pres sure to desired levels in relation to atmospheric pressure By changing the height of the reservoir all parts of the muscle vascular bed could thus be exposed to graded increase or decreases in vascular transmural pressure (P_T) whi h could be applied at any desired rate dP_T/dt without interference with the arterio-venous pressure differenc This pl thysmographic technique also permitted recordings of tissue volume variations

Recordings of total and segmental vascular resistances in the muscle region Vascular resisten es were determined as the ratio: mean perfusion pressure/ mean arterial blood flow (Q) and expressed in peripheral resistan units PRU (mm Hg ml^{-1} min 100 g tissue) The following definitions were used total vascular resistance (R_{tot}) (AP VP)/Q; proximal arterial resistance (R_{prox}) (AP - SAP)/Q; microvascular resistance (R_{micro}) (SAP SVP)/Q; and large v in resistance (R_{ven}) (SVP - VP)/Q In most cases continuous recordings of the e vascular resistances were obtained with the aid of resistan e meters (ee next chapter) In some cases resistance was calculated ma nually All vascular parameters were recorded on a ten channel Grass Polygraph

The distinction b tween proximal arterial vessels microvessels and lar g veins ref rs to the it from which SAP and SVP were monitored The used method to measure these pressures is indirect and the recordings represent microvascular pressures as transmitted via communicant vessels from the micro circulation to the distal ramifications of the sural artery and vein Arguments were given (papers II and VI) for the validity of thi approach to measure microvascular pressures in the studied muscl region

Especially useful for the interpretation were some recent vital microscopy observations f the size of the parent arterial vessels at the site from which

the most proximal communicants emanate (20 - 25 µm i d ; e g Eriksson and Myrhage 1972) and further some data on simultaneous vascular diameter and micropipette pressure measurements along the vascular tree in skeletal muscle (e g Fronek and Zweifach 1975) It can be concluded that BAP in all the present investigations was monitored from arterial microvessels with an approximate average inner diameter of about 25 µm implying that the so called 'proximal arterial vessels' were larger than and the 'arterial microvessels smaller than this dimension (the size of 15 - 20 µm stated in paper II and III seems to be an underestimation)

The site from which SVP was recorded is more difficult to precisely define due to the quite flat pressure drop curve across the venous microvessels (Fronek and Zweifach 1975) Approximate deduction suggests that SVP was monitored from venoles of about 20 40 µm (i d) Despite the fact that the capillaries and the minute veins were included in the microvascular resistance recordings the myogenic reactions to be described in all probability occurred mainly on the precapillary side as discussed for instance in paper II

Correct segmental resistance measurements presuppose that arterial blood flow is uniform throughout the vascular bed It was concluded (paper II) that this demand was reasonably well fulfilled under most experimental conditions in th present investigations with the possible exception for the large vein section during phasic change of a vascular transmural pressure when larg vein resistance recordings might be somewhat unreliable

From these and other methodological considerations presented in the investigations it may be concluded in summary that the experimental approach used can be considered to provide useful and reliable observations of myogenic reactivity to transmural pressure stimuli in the whole vascular bed of skeletal muscle as well as in its segments the proximal arterial vessels and the microvessels and with some reservation also the large veins

DESIGN OF AN ELECTRONIC DIFFERENTIAL PRESSURE FLOWMETER AND A RESISTANCE METER FOR CONTINUOUS MEASUREMENT OF VASCULAR RESISTANCE

(paper I)

The aim of the present investigations was to study dynamic and static myogenic reactivity to phasic and steady-state changes of vascular transmural pressure in the vascular bed of the lower leg muscles in the cat, and to differentiate between the responses in proximal arterial vessels microvessels and large veins Such studies require specialized technical instrumentation which permits accurate and continuous recordings of blood flow in a range as small as 1 ml/min and further reliable continuous and simultaneous recordings of segmental vascular resistance responses even during short lasting phasic shifts of vascular transmural p essure Most conventional flowmeters fo continuous measurement like the electromagnetic one show poor technical characteristics in this low flow range A special flowmeter and a vascular resistance meter were therefore designed which satisfied the above-mentioned demands

The flowmeter was based on the differential pressure principle This principle has been applied in several previous flowmeter designs (e g De Burgh Daly 1926 Lawson and Holt 1939 Ueno and Takenaka 1955) but has been of little practical use in biological research because of several inherent problems Thus the very small pressure fall over the flowmeter created by flow has been difficult to ecord exactly with the so far used mechanical optical or strain gauge manometers The previous instruments have also suffered from alinearity and considerable problems related to the phase lag of the arterial pulse pressure etc

Recent developments in the fi ld of piezo-resistive pressure transducers especially the hybrid IC type (National Semic Corp) offer the possibility of precise and reliable measurements of minute differential pressures The present flowmete in which such a transd cer was used and in which the problems with the pulse pressure phase lag were largely overcome was shown to provide a means for reliable and linear ecordings of mean (or pulsatile) blood flow even in the small flow range encountered in the studied cat skeletal muscle region

Fig 1 illustrates the flowmeter principle (left pa t) in combination with a resistance meter (ight part see below) as adopted for the present measurements of total and segmental vascular resistances in the skeletal muscle region

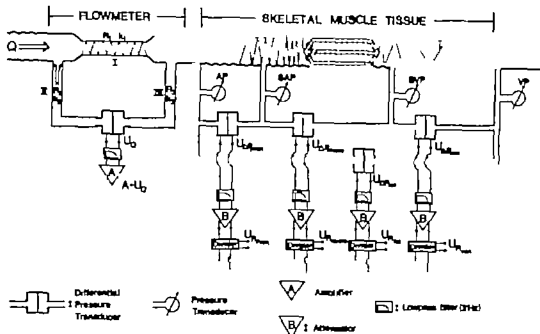


Fig 1 Schematic illustration of the differential pressure flowmeter (left part) and the resistance meters (right part) as adopted for the present studies on the vascular bed of skeletal muscle The flowmeter consists of tubes I II and III where R denotes tube resistance k the tube elasticity modulus and U_Q the flowmeter signal The resistance meters consist of differential pressure transducers giving pressure signals $U_{\Delta P}$ and of electronic divider circuits providing continuous recordings of total resistance ($U_{R_{tot}}$) proximal arterial resistance ($U_{R_{prox}}$) microvascular resistance ($U_{R_{micro}}$) and large vein resistance ($U_{R_{ven}}$)

The flowmeter consists of a main tube (I) across which blood flow is diverted from the proximal to the distal end of the severed artery The pressure drop over this tube is measured with a differential pressure transducer with virtually no membrane displacement via tubes II and III It was highly essential that pulsatile pressure was transmitted to either side of the differential pressure transducer without any significant phase lag The latter was accomplished by choosing appropriate values for the tube resistances R_1 R_2 and R_3 as well as for their elasticity moduli k_1 k_2 and k_3 (Fig 1) It was calculated that no phase lag at zero flow would exist if the relation $R_2/k_2 = (R_3 + R_1)/k_3$ was satisfied This was also experimentally confirmed by the absence of arterial pulse pressure oscillations during occlusion of the artery just distal to the entrance of tube III Further tests (unpublished) have demonstrated that accurate mean flow recordings were achieved with this flowmeter for flows in the range from zero up to 20 ml/min for pulsation frequencies up to 4 Hz at normal pulse pressure amplitude

Since blood flow is diverted extracorporeally on the arterial side in tube I of the flowmeter the tube was made of an inert silicone rubber material moulded in such a way that the inner surface of the tubing was entirely smooth Test experiments demonstrated that this flowmeter ca sed no hemolysis nor any release of vasoactive substancies the latter evidenced by virtually unchanged vascular resistance in the steady-state after insertion of the flowmeter circuit

This flowmeter was used in all the present investigations and was found to be linear in and well above the flow range encountered in these studies Base line drift was small

The vascular resistance meters (see Fig 1 right part) consisted of electronic divider curcuits into which were fed the signals from differential pressure transducers which recorded the pressure drop across the whole vascular bed and its segments and the signal from the arterial flow meter

RESULTS AND COMMENTS

Static and dynamic myogenic reactivity in the vascular bed of skeletal muscle (papers II and III)

In most previous investigations in vivo myogenic reactions have been studied in response to change of arterial and/or venous pressure The interpretation of myogenic responses to such changes of intravascular pressure is complicated by the fact that induced passive alterations of blood flow lead to simultaneous interference with the metabolic vascular control system With the present ap proach in which all parts of the vascular bed were exposed to change of vascular transmural pressure (P_T) by variations of extravascular (plethysmographic) pressure such flow induced influences of the metabolic control system were largely avoided since the arterio-venous pressure difference was maintained constant

To reveal a possible dynamic or rate-sensitive component in the myogenic con trol besides the previously described static component vascular resistance changes in the sympathectomized skeletal muscle were studied in response to a given increase or decrease in vascular transmural pressure P_T applied at dif ferent rates dP_T/dt The resistance responses were thus followed both during the dynamic phase of the transmural pressure change and in the steady-state (static) phase of constant increased or decreased pressure

In a first series of expts (paper II) such responses were studied in the whole vascular bed as well as in its segments the proximal arterial vessels the microvessels and the large veins (for definitions see Methods) The expts were performed on the normal myogenically reactive as well as on the completely paralysed (papaverine-dilated) vascular bed Vascular transmural pressure in this study was always changed by a total of 40 mm Hg but the pressure change was applied at two distinctly different rates viz in 15 s and 120 s corresponding to dP_T/dt values of 2 7 and 0 33 mm Hg/s (positive and negative)

The results from these expts are summerized in Fig 2 The paralysed vascular bed (dotted curves) showed as expected an entirely passive behaviour in terms of decreased resistance in all vascular sections during the whole period of in creased transmural pressure In the vascular bed with preserved basal tone

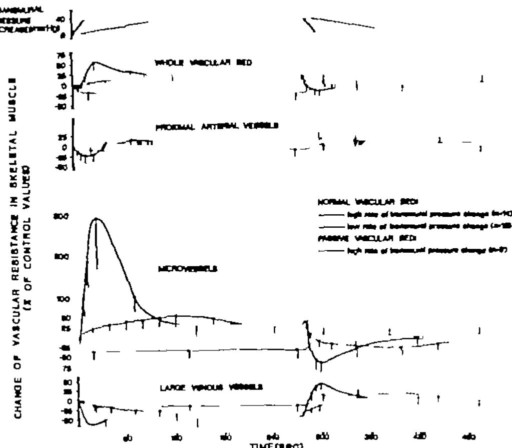


Fig 2 Compiled data for total and segmental vascular resistance changes in the normal myogenically reactive and in the passiv myogenically inactive sympathectomized vascular bed of skel tal mus le voked in respons to increased and subsequently decreased vascular transmural pressure (40 mm Hg) applied at high (2 7 mm Hg/) and low (0 33 mm Hg/s) rates Note the presence of both a rate-dependent dynamic and bi-directional component and a static component in the myog nic microvascular response and the passiv behaviour in the paralysed vascular bed

(solid and dashed curves where the former show the responses to the high and the latter to the low rate of the transmural pressure change) a passive dis tension eff ct of the transmural pressure rise was maintained only in the large vein section but was very transient in the others Thus within few seconds after the beginning of the pressure increase resistance rose to exceed the control value in the whole vascular bed and in the microvessels and with some longer delay also in the proximal arterial vessels demonstrating active constrictor responses in these sections to the tra smural pressure stimulus In the microvascular section the constrictor responses reached high peak va lues shortly after completion of the transmural pressure rise and these effects were also reflected in overall regional vascular resistance The magnitude of these peak constrictor responses were however much greater with the high (2 7 mm Hg/s) than the low (0 33 mm Hg/s) rate of the transmural pressure increase

The later steady state constrictions in response to constant increased trans mural pressure on the other hand were small and of equal size at both rates When transmural pressure was decreased back to the control level at the same specified rates (dP_T/dt 2 7 and 0 33 mm Hg/s) the pattern of response was reversed with pronounced active microvascular dilation far below the control vascular tone in the period of phasic decline of pressure These responses were also clearly graded in relation to the rate of the pressure decline Vascular tone then gradually returned to the initial control level These microvascular dilator responses were also reflected in overall vascular resistance The de scribed pattern of vascular response was entirely unaffected by adrenergic blockade (phenoxybenzamine and propranolol) strongly indicating that the reac tions were of local myogenic origin

These results demonstrate the existence of a clearcut two-component effector *response in the microvessels to altered vascular transmural pressure The* initial component developing during the period of changing pressure was much larger when evoked by high than low rate of transmural pressure change strongly indicating that the response was rate-dependent The subsequent steady state component revealed during the period of constant increased pressure was small and rate independent This microvascular response pattern after exclusion of some alternative explanations (see paper II) was interpreted to demonstrate the existence of a dynamic rate-sensitive as well as a static component in the myogenic response of the microvessels to changed transmural pressure (dP_T/dt and P_T respectively) in skeletal muscle This conclusion was strongly sup- ported by the close resemblance between this pattern of microvascular response and the analogous pattern response of the rat portal vein to passive static stretch and shortening applied at various rates the latter study providing *unequivocal evidence for dynamic and static* myogenic vascular reactivity (see introduction)

The rate-dependent microvascular constrictor response at the dP_T/dt value of 2 7 mm Hg/s (Fig 2) was 10 15 times greater than the static steady-state response It was concluded that the microvessels in muscle exhibit strong myogenic reactivity to transmural pressure stimuli though the behaviour was dominated by the dynamic rather than the static characteristics of the stimu lus The proximal arterial vessels on the other hand seemed to exhibit on ly static myogenic reactivity to a transmural pressure change (Fig 2) where- as the large vein section which responded passively *appeared to lack myoge* nic reactivity entirely

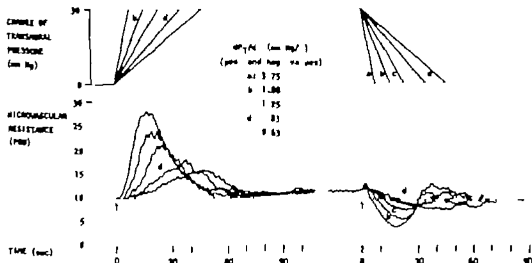


Fig 3 Dynamic and static myogenic microvascular re istance responses in sym pathectomized skeletal muscle evoked by increase and a subsequent decrease in vascular transmural pressure (P_T) by 30 mm Hg applied at 5 different rates dP_T/dt (see k y)

More detailed information about the stimulus-effector characteristics of the dynamic and static components in myogenic microvascular control was obtained from another series of expts (paper III) The analysis was here performed du ring graded changes of static vascular transmural pressure (P_T) from 0 to 50 mm Hg applied at different rates dP_T/dt in the entire range from +7 5 to 7 5 mm Hg/s

Fig 3 illustrates in a representative expt how microvascular resistance in skeletal muscle was influenced by a given total increase in P_T of 30 mm Hg applied at five diffe ent rates in the dP_T/dt range from 0 63 to 3 75 mm Hg/s and by subsequent decrease in P_T back to the control level at the same given (egative) rates To permit direct comparison the microvascular resistance responses to different dP_T/dt stimuli have been eplayed on top of each other in this figure Change of P_T led to the following general pattern of microvascu lar resistance response Few seconds after the onset of the P_T rise resistance sta ted to increase and reached a peak or transient plateau shortly after P_T had attained the final constant level of 30 mm Hg above the control value Re sistance then declined again despite the fact that static t ansmural pressure was maintained at the high constant level and it stabilized at a steady-state level only a few resistance units above the initial control value When P_T was decreased agai the microvessels showed clearcut active dilator responses graded in relation to the rates of the pressure decline The subsequent reco very of microvascular tone was characterized by an overshoot or an oscillatory

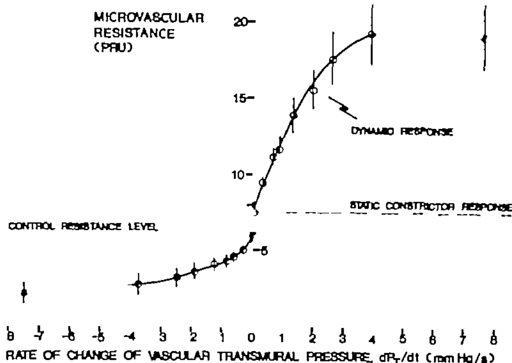


Fig 4 Diagram illustrating the average dynamic myogenic microvascular constrictor and dilator responses in skeletal muscle to increased and subsequent decreased vascular transmural pressure by 30 mm Hg applied at various rates from +7 5 mm Hg/s to -7 5 mm Hg/s (n 7) *The average static constrictor response* to constant increased P_T by 30 mm Hg above the control level is also shown

behaviour before resistance stabilized at its initial control value

These results further strengthen the view of a rate-sensitive dynamic and a *static regulatory mechanism in myogenic microvascular control and show in* greater detail that the rate-sensitive mechanism in operation during the phase of the transmural pressure change is bi-directional causing graded excitatory constrictor effects in response to positive and graded inhibitory dilator effects in response to negative values of dP_T/dt Note that the static myogenic response revealed during the phase of steady-state constant increased transmural pressure was quite small compared to the dynamic responses

Fig 4 summarizes the results for the average dynamic microvascular constrictor and dilator responses to dP_T/dt stimuli over the range from +7 5 to 7 5 mm Hg/s The average static constrictor response to the standardized increase in P_T by 30 mm Hg is also illustrated It can be seen that the microvessels exhibited smoothly graded dynamic constrictor responses to excitatory dP_T/dt *stimuli (pressure increase) in the lower range of test with a maximum reached* at a dP_T/dt value of about +4 mm Hg/s At higher rates the resistance curve

levelled off demonstrating that extreme myogenic effector reactions are not elicited Similarly there were graded active microvascular dilator responses to inhibitory dP_T/dt stimuli (pressure decrease) maximal dilator effects being reached at the dP_T/dt value of -4 to 5 mm Hg/s Further analysis indicated that the resistance change per unit time dR_{micro}/dt was also graded in relation to dP_T/dt both for positive and negative values

The static myogenic microvascular responses were shown to be graded in relation to the amplitude of the P_T increase within the range up to about 40 mm Hg above normal transmural pressure The static transmural pressure stimulus when maximal increased microvascular resistance by only about 2 PRU above control which should be compared with the effect of a strong dynamic transmural pressure stimulus which was 10 15 times more effective in exciting the smooth muscle of the microvessels

The size of the dynamic microvascular constrictor response though basically dependent on the magnitude of the dynamic stimulus dP_T/dt also seemed related to the duration of the latter and/or the amplitude of the static transmural pressure change (paper III) This quite complex effector response was tentatively explained by gradual recruitment of an increasing number of dynamic receptor u its with different thresholds as P_T rises A recent mathematical analysis of myogenic microvascular reactivity (Borgström and Grände 1979) suggested that this phenomenon alternatively or in addition could be ascribed to a increase in gain implicit in the inverse fourth power relationship between v scula resistance and vessel radius according to the law of Poiseuille

The approach used in the present investigation by which myogenic reactivity was studied during equal increase of transmural pressure in all sections of the vascular bed permits basic description of the characteristics of its static and dynamic components the latter in terms of the transient effector response to a short lasting phasic transmural pressure change This experimental approach resembles the hemodynamic in vivo situation in dependent vascular beds when exposed to hydrostatic load as created for example upon shift from the supine to the erect body posture The initial load on the dependent vascular bed caused by kinetic energy then exceeds sev ral times the later steady-state hydrostatic load and the very marked microvascular resistance increase noted in the present expts (Fig 2 and 3) might then be needed to protect the vessels against damage It is quite likely however that the described rate-sensitivity plays a mo e universal role in myogenic vascular control with biological

implications analogous for instance to those in the muscle spindle of skeletal muscle *with its highly differentiated* dynamic and static receptor organization (e g Matthews 1964) The rate-dependent regulatory mechanism in myogenic circulatory control might serve the important function to increase its rapidity stability and sensitivity like in many other servo control systems (see General Discussion)

Role of intravascular static and dynamic transmural pressure stimuli in the development of normal basal vascular tone
(paper IV)

The previous section was concerned with myogenic vascular responses to experimentally applied changes in vascular transmural pressure In the expts to be described in this section an attempt was made to determine to what extent normal intravascular pressure on the arterial side of the muscle vascular bed via static and dynamic transmural pressure stimuli might be responsible for the initiation and maintenance of normal myogenic basal vascular tone For this purpose the characteristics of the intrinsic basal vascular tone in the adrenergically blocked skeletal muscle were analysed with regard to its extent and site along the vascular bed its dependence on static mean arterial blood pressure distension and dynamic pulse pressure distension and finally its sensitivity to local metabolic influence The expts were designed in such a way that by analogies to electrophysiological studies of myogenic reactivity in other vascular smooth muscle in vitro some tentative considerations could be given about the cellular events behind basal vascular tone

The study first confirmed previous observations that basal vascular tone in skeletal muscle which apparently is of myogenic nature (e g Folkow 1962) was pronounced in proximal arterial vessels and in microvessels but low in *large veins* *As a first approach* to the problem of its underlying mechanisms a series of expts was performed to reveal the extent to which normal basal vascular tone on the arterial side can be decreased by a maximal vascular transmural pressure stimulus of inhibitory nature For this purpose the vascular bed at normal intravascular distending pressure was exposed to a strong negative dP_T/dt stimulus known from the data in the previous section to cause effective vasodilation

The results demonstrated that the applied transmural pressure stimulus (P_T decline of 40 mm Hg applied at the rate of -5 mm Hg/s) was capable to very effectively inhibit basal tone in the microvessels where resistance decreased by almost 90 % of that evoked by papaverine the latter substance known to entirely abolish vascular tone whatever its cause Such an inhibitory stimulus however was much less effective in the proximal arterial vessels the basal tone (PRU) of which could be decreased by no more than about 10 % of the papaverine effect Evidently basal tone in the microvessels is much more sensitive to the mechanism which mediating the response to an inhibitory transmural pressure stimulus than that of the more proximal vessels suggesting that basal tone in these consecutive vessels is of somewhat different nature

In a second series of expts the importance of normal arterial blood pressure pulsations on basal vascular tone was investigated For this purpose, total and segmental vascular resistances in the adrenergically blocked muscle region were analysed at normal basal tone upon shift from pulsatile to non-pulsatile perfusion under maintenance of a normal and constant mean arterio-venous pressure difference The study demonstrated, that such elimination of the arterial pulse pressure oscillations caused a pronounced inhibition of basal tone in terms of a decrease by almost 20 % in total vascular resistance in the muscle confirming previous results of Hellander and Arvidsson (1974) and LaLone (1975) The major part of this dilator response however was shown to be localized to the microvessels the resistance of which decreased by almost 35 % below control whe eas resistance in the mo e proximal arterial vessels decreased by only some 10 % It was concluded, that the normal arterial pulse pressure oscillations seem to cont ibute significantly to the development of basal tone though mainly in the microv ssels an effect attributed to repetitive positive dynamic transmural pressure stimuli on the vascular smooth muscle evoked during the rising phase of the pulse pressu e curve

In a thi d series of expts an attempt was made to reveal to what extent arterial blood pressure via its static mean pressure distension effect might contribute to the initiation and maintenance of basal vascular tone For this purpose changes of basal vascular tone in the 'proximal arterial vessels and the microvessels were analysed in response to decreases in arterial blood pressure and fu ther in response to superimposed restoration of mean distending pressure to normal under maintained arterial hypotension This restoration of vascular transmural pressure was performed by decrease of extravascular pressu e Special tests were performed to reveal possible simultaneous influence by the induced arterial hypotension It was concluded from

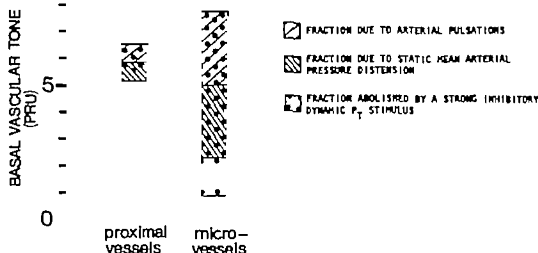


Fig 5 Histogram summarizing in approximate figures the extent of normal basal tone in proximal arterial vessels (>25 µm) and microvessels (<25 µm) in skeletal muscle (total height of columns) and its dependence on transmural pressure stimuli (see key) Note that a considerable fraction of the normal basal tone in the microvessels is dependent on arterial pressure both via its static distension effect related to mean arterial pressure and its dynamic pulse pressure distension Note further that a strong inhibitory dynamic transmural pressure (P_T) stimulus (dP_T/dt -5 mm Hg/s) could virtually abolish basal tone in these vessels Basal tone in the proximal arterial vessels was much less affected by such transmural pressure stimuli

different expts that normal arterial blood pressure via its static mean pressure distending effect significantly contributes to the development of basal tone on the arterial side though more markedly in the microvessels than the proximal arterial vessels In rough figures static mean arterial pressure distension seemed to be responsible for some 35 % of the normal basal tone in the former but only for some 10 % in the latter

The results of these investigations can be summarized schematically and in rough figures as illustrated in Fig 5 The extent of smooth muscle tone on the arterial side in the proximal arterial vessels (>about 25 µm i d) and the microvessels (<about 25 µm) is here expressed in terms of its active vascular resistance corollary (PRU) i e the resistance at the normal level of basal tone minus the small physical resistance remaining after complete smooth muscle relaxation induced by papaverine Under control conditions basal tone corresponded to an average of 6 6 PRU in the proximal arterial vessels and 7 8 PRU in the microvessels The normal arterial blood pressure thus seemed to be partially responsible for the initiation and maintenance of this basal tone both via its pulse p essure oscillations and its mean pressure level the former apparently creating a repetitive dynamic and the latter a static trans

mural pressure stimulation of the smooth muscle These two stimuli were about equally effective The figure might illustrate more directly that the fraction of basal tone which could be attributed to these two stimuli was much greater in the microvessels than in the proximal arterial vessels Experimental ap plication of a seemingly maximal inhibitory dynamic transmural pressure stimu lus (dP_T/dt = 5 mm Hg/s) caused a very effective still not complete inhibition of the normal basal tone in the microvessels but such a stimulus had only a slight effect on tone in the more proximal arterial vessels

The results of this study which in different ways demonstrate that basal tone is much more dependent upon and sensitive to transmural pressure stimuli in the microvessels than in the proximal arterial vessels strongly suggest that basal tone is of somewhat different nature in these two vascular sections The functional behaviour reported for the microvessels (Fig 5) might in fact refer to the entire microcirculation to which the smallest ramification of the proximal arterial vessels most likely belong in view of the somewhat arbitra ry delineation between proximal arterial vessels nd microvessels with the present approach viz at the vessel dimension as small as about 25 µm

Even if basal tone in both the arterial microvessels and the more proximal ar terial vessels in skeletal muscle by conventional definition can be considered to be of myogenic origin (e g Folkow 1962) the described functional discre pancies between the two sections (Fig 5) might suggest somewhat different cel lular mechanisms behind their basal vascular tone Circumstantial evidence will be presented in the General Discussion section for the tentative view that vas cular smooth muscle in the rterial microvessels is mainly of the spike-genera ting type and that in the somewhat larger arterial vessels more of the non spike-generating contract e type

Adrene gic influence on myogenic mic ovascular reactivity in skeletal muscle (papers V and VI)

The concept of myogenic vascular control has been much debated since it was ori ginally proposed by Bayliss (see Introduction) One of the reasons has been that even seemingly well designed expts can exhibit poor or occasionally fai ling myogenic reactivity Such expe iences must not necessarily be taken as evidence agai st the myogenic hypothesis which occasionally seems to have been the case but might merely imply that the myogenic vascular control system by some mechanism is vulnerable to expe imental intervention

This problem was approached in paper V which revealed that stress induced release of catecholamines from adrenal medulla via its β adrenergic influence could explain much of the decreased myogenic reactivity under experimental conditions The evidence was as follows The sympathectomized vascular bed of skeletal muscle exhibited impaired dynamic myogenic reactivity and decreased microvascular tone in the early period after completion of surgery but gradual recovery towards normal within about an hour of subsequent rest These findings indicated the presence of some inhibitory factor(s) which gradually disappeared during extended rest and therefore most likely was activated during the surgical and other experimental intervention Early post-surgical β-adrenergic blockade with propranolol caused almost immediate restoration of myogenic reactivity and of vascular tone towards normal indicating that the inhibitory effect to a great extent was evoked by β adrenoceptor stimulation of circulating catecholamines Poor myogenic reactivity reported in some previous investigations on more or less traumatized preparations might thus simply be explained by stress induced β adrenergic inhibitory interaction with the myogenic control system

The interaction between adrenergic and myogenic regulatory mechanisms was analysed more systematically in paper VI in an attempt to reveal in particular whether the β adrenergic vascular control system might serve the function to modify myogenic reactivity also under normal physiological conditions For this purpose myogenic vascular reactivity to a standardized transmural pressure stimulus (P_T increase of 40 mm Hg applied at a constant ate of 2 5 mm Hg/s) was studied in skeletal muscle before and during graded neural and humoral adrenergic influence on the ascular bed within the physiological range

Fig 6 shows effects of graded sympathetic nerve stimulation (0 12 Hz) on microvascular resistance (abscissa) and on the superimposed dynamic myogenic constrictor response (ordinate) to the standardized test transmural pressure stimulus on the muscle vascular bed with I tact α and β adrenoceptors (lower curve) *and after effective β adrenergic* blockade (upper curve) Sympathetic nerve stimulation caused as expected an increase in microvascular tone (abscissa) graded in elation to the excitation rate both on the intact a d the β-blocked vascular bed This constricto response was somewhat less pronounced in the former especially at high stimulation rates I dicating an inhibitory β adrenergic microvasc lar resistance effect of neural excitation in the intact vascular bed which confirms previous results by Lundvall and Järhult (1976) The superimposed myogenic microvascular co strictor response to the

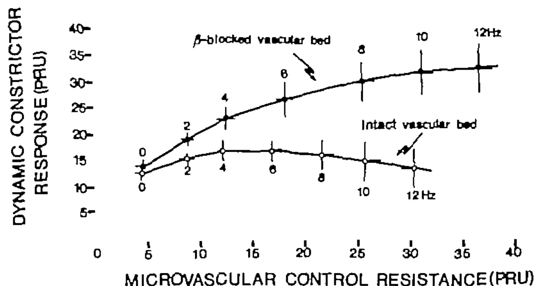


Fig 6 Effects of graded sympathetic nerve stimulation on microvascular resis tance (abscissa) and on the superimposed dynami myogenic constrictor response (ordinat) to a standardized test transmural pressure stimulus on the vascular bed with intact adrenoceptors (lower curv) and after effective β-adrenergic blockade (upper curve)

test stimulus (o dinate) was of almost the same magnitude at all rat s of sympathetic stimulation in the intact vascular bed but significantly increased when the β-adrenoceptors were blocked Since the neural α adrenergic influence can be considered equal in the two series of expts the depicted significantly smaller dynamic constrictor response in the intact vascular bed must be ascribed to a neural β adrenergic inhibitory influence on myogenic reactivity It was concluded that sympathetic nerve activation via a β adrenergic compo nent exerted an effective inhibitory action on dynamic myogenic microvascular reactivity graded in relation to the nerve excitation rate Such graded β adrenergic inhibitory effects were also revealed during infusions of adrenaline and noradrenaline where adrenaline was shown to be the more effective inhibi tor and capable to decrease the myogenic response well below the control level

The quite remarkable effect demonstrated in Fig 6 in terms of gradually increa sing dynamic myogenic constrictor responses in the β blocked vascular bed du ring sympathetic nerve stimulation (and also during catecholamine infusion) did not seem to be explained by reinforcement of myogenic reactivity by α adrenoceptor stimulation As will be discussed it more likely can be ascribed to a physical effect of the concomitantly aised microvascular tone leading to an i crease in gain implicit in the inverse fourth power relationship between microvascular resistance and internal vessel radius according to Poiseuille s

law Since this reinforcing phenomenon was not observed on the vascular bed with intact adrenoceptors (see Fig 6) it was suggested that the β adrenergic mechanism in neural (and humoral noradrenergic) sympathetic control serves the seemingly important function to compensate for such a physical effect, implying the development of an almost equally large myogenic resistance response to a given transmural pressure stimulus irrespective of the prevailing level of vascular tone (see General Discussion)

The described even more pronounced inhibitory effects of adrenaline might be encountered under in vivo conditions only during relatively severe stress and could then serve the function to facilitate capillary exchange by decrease of myogenic tone and reactivity in resistance vessels and precapillary sphincters (cf Lundvall and Hillman 1978)

GENERAL DISCUSSION AND SUMMARY

The studies summarized in this paper deal with the active smooth muscle responses of the vascular bed of skeletal muscle to the mechanical force exerted by intravascular or transmural pressure Such locally mediated changes of vascular tone have by convention been denoted myogenic reactivity The experimental approach was such as to permit observations of myogenic responses without significant simultaneous interference with the local metabolic control system This was accomplished by exposing the whole vascular bed under study to changes of vascular transmural pressure via variations in extravascular pressure thus avoiding alterations of the perfusion pressure and hence passive changes in blood flow With this technique the vascular bed could be exposed to graded change in vascular transmural pressure (P_T) both with regard to its amplitude (static transmural pressure stimulus) and its rate dP_T/dt (dynamic transmural pressure stimulus) The experimental design allowed simultaneous and continuous recordings of vascular resistance in the whole vascular bed as well as in its longitudinal segments the proximal arterial vessels ($>$about 25 µm i d) the microvessels ($<$about 25 µm) and the large veins

The results were interpreted with regard to their most direct physiological implications in the foregoing sections Their possible functional significance will be discussed below in a wider and therefore perhaps somewhat more speculative perspective

The data in papers II and III demonstrated the existence of a dynamic or rate sensitive component in addition to the previously known static component in the myogenic vascular response to changed transmural pressure Rate-sensitivity in myogenic control however seemed to be present only in the microvessels of skeletal muscle whereas static myogenic reactivity was present also in the proximal arterial vessels The large veins on the other hand seemed to be devoid of myogenic reactivity Analysis of the stimulus-effector characteristics in microvascular myogenic control revealed that the dynamic resistance responses developing during the phase of changing P_T were pronounced and distinctly graded in relation to the magnitude of the dP_T/dt stimulus both with regard to the amplitude of the resistance response and the rate of the resistance change per unit time Moreover rate-sensitivity in myogenic microvascular control was found to be bi-directional eliciting excitatory effects (con-

striction) in response to positive and inhibitory effects (dilation) in response to negative values of dP_T/dt Maximum dynamic constrictor responses were obtained at a dP_T/dt value of about +4 mm Hg/s and maximum dilator responses at a negative dP_T/dt value of about the same order of magnitude With stronger dynamic transmural pressure stimuli the effector responses levelled off The static responses revealed in the steady-state phase of constant increased P_T were comparatively small and graded in relation to the amplitude of the P_T increase A strong dynamic transmural pressure stimulus was some 10 15 times more effective in increasing microvascular tone than a strong static stimulus

It is evident that the dynamic component in myogenic microvascular control will serve the function to accomplish very prompt effector responses to a given transmural pressure change There is much to indicate however that rate-sensitivity is of importance not only for such transient effector responses but might be of more generalized physiological significance in myogenic vascular regulation A rate-dependent regulatory mechanism especially when bi-directional is of great importance in many servo-control systems in order to increase rapidity stability and sensitivity and thereby regulatory efficiency The described biological data taken together indicate that the dynamic component in myogenic microvascular control subserves such purposes For proper function most servo-control systems require a smoothly graded relation between the rate-dependent input signal and the resulting output signal (Heize and Schultz 1969) Such relations were demonstrated for the dynamic transmural pressure stimulus and its effector response the latter in terms of microvascular resistance change as well as rate of the resistance change per unit time This also applies to stretch induced dynamic myogenic reactivity in the rat portal vein and its underlying change of pacemaker generated spike discharge

More detailed information about the quite complex variables involved in myogenic vascular control in vivo might be derived from a recent matematical analysis of the described static and dynamic myogenic responses in the microvessels to a given change of transmural pressure applied at various rates (Borgström and Grände 1979)

This mathematical model was based on a force-equilibrium equation including passive forces related to vascular transmural pressure elasticity and wall viscosity and the active myogenic forces related to the mentioned static and dynamic responses to change of transmural pressure It was assumed in the approach that the myogenic responses were related to and triggered by changes of

wall tension an opinion tentatively suggested in some previous publications (Johnson and Wayland 1967 Sigurdsson Johansson and Mellander 1977) Great resemblance was demonstrated between the microvascular resistance curves obtained with this model and the corresponding curves presented in paper III of the present study indicating that the model quite adequately can describe myo genic microvascular resistance responses to transmural pressure stimuli These results supported the myogenic hypothesis in general and in particular the concept of an important rate-sensitivity in myogenic microvascular control They strongly indicated that microvascular myogenic reactions are triggered by and related to change of wall tension i e to σ (static response) and to $d\sigma/dt$ (dynamic response) The mathematical model predicted that σ increases above control during a transmural pressure increase implying a tendency of a positive feedback in myogenic control However after an initial rise $d\sigma/dt$ declined implying an inherent negative feedback in myogenic regulation which significantly can help to stabilize this circulatory control system

A tentative description of myogenic autoregulation was presented by Mellander (1978) in which vascular tone during increased transmural pressure was sugges ted to oscillate around an increased level as a result of mainly repetitive dynamic transmural pressure stimuli on the vascular smooth muscle A damped oscillatory behaviour of microvascular tone was a characteristic finding after a fast change of vascular transmural pressure (paper II) or of arterial pres sure (paper IV) when myogenic reactivity was well preserved Oscillatory res ponses are typical for vasomotion and have also been reported in some previous hemodynamic studi (e g Folkow 1953 Stainsby and Renkin 1961) though with out convincing explanations It is known however that control systems may develop an oscillatory behaviour if a first derivative (rate-dependent) component as well as higher order derivative component(s) are present (Helza and Schultz 1969) Although not specifically analysed in the present papers a second (or higher order) de ivative component is present in myogenic control due to biological inertia to time delay and to the presence of actionpotential afterdischarge the latter evidenced for myogenic reactivity in vitro (Johansson and Mellander 1975) Incorporation of such characteristics in future theories of myogenic regulation might lead to much-improved understanding of the mode of operation of this control system

It has sometimes been invoked that the myogenic control system could be self limiting insofar that a myogenic smooth muscle shortening in response to raised distending pressure automatically would abolish the primary receptor stimulus

If the receptors were sensitive to change in length (e g Gaskell and Burton 1953) Such self limitation is less likely to occur with myogenic receptors sensitive to wall tension and its rate of change dσ/dt being largely indepen dent of absolute muscle length

Myogenic reactions to transmural pressure stimuli are effected by increases or decreases of the normal myogenic basal tone In an attempt to get deeper in sight into the interaction between myogenic reactions and basal tone the characteristics of the latter were investigated in some detail (paper IV) These studies revealed that normal basal tone in the microvessels seemed to be inti mately dependent on the arterial blood pressure level and to a significant ex tent (some 70 %) initiated and maintained by the combined effects of its static mean pressure distension and its dynamic pulse pressure distension Microvascu lar tone could be virtually abolished by a transmural pressure decrease applied at fast rate i e by a strong inhibitory dynamic transmural pressure stimulus Basal tone in the proximal arterial vessels on the other hand was little affected by arterial pressure and almost irresponsive to inhibitory dynamic transmural pressure stimuli Another difference was that basal tone in micro vessels was much more sensitive to metabolic stimuli than that in the proximal arterial vessels It was concluded that basal tone in the arterial micro vessels and in the more proximal arterial vessels at least in part is of quite different nature

The demonstration that normal microvascular basal tone to such a significant extent is depending upon static and dynamic transmural pressure stimuli crea ted by the normal arterial blood pressure might offer a very simple explanation of myogenic autoregulatory phenomena in the microcirculation Fall of arterial blood pressure within a certain range would lead to a graded inhibition of basal tone simply due to partial elimination of its initiating static and dynamic pressure stimuli Conversely raised arterial pressure might lead to re inforced vascular tone due to increased static mean pressure distension and to the usually associated increase in the amplitude and/or frequency of the pulse pressure oscillations The fact that basal tone in the proximal arterial vessels was largely independent of a terial blood pressure per se might then possibly explain why auto egulation of flow occurs only within a limited range of arterial pressures

Mention should be made in this context that dynamic transmural pressure stimu lation of the vascula bed might not solely be affected by pulse pressure oscil

lations but perhaps also by segmental pressure oscillations in the longitudinal direction created for instance by the intermittent local constrictions and dilations denoted vasomotion

It appears that the observations made in paper IV might help to shed some light on the cellular mechanisms responsible for basal vascular tone and myogenic reactions These mechanisms are not yet experimentally defined in the arterial microvessels specifically Howeve the recent in vitro study by Johansson and Mellander (1975) mentioned in Introduction has clarified the electrophysiological events behind myogenic reactivity to mechanical stimuli in the single unit spike-generating vascular smooth muscle of the rat portal vein which seems to have several characteristics in common with arterial microvascular smooth muscle The myogenic contractile response pattern is thus quite similar in both these types of vascular smooth muscle Both exhibit a static as well as a dynamic component in their myogenic responses of which the latter is the much more prominent one; a maximal excitatory dynamic stimulus was thus some 15 times more effective in causing contraction in vitro and microvascolar constriction in vivo than a maximal static stimulus Furthermore a strong inhibitory dynamic stimulus was capable to abolish contractile activity in the portal vein and as shown in paper IV also could effectively inhibit basal microvascul r tone in vivo Such close resemblance between the portal vein and the arterial microvessels with regard to their myogenic contractile patterns of response and stimulus-effector characteristics makes it quite likely that the myogenic responses in both types of muscle are mediated via some common cellular mechanism

The portal v in shows a characteristic spontaneous *regula rhythmic contractile* activity in the control state at low preload caused by bu sts fo action potentials primarily generated from a pacemaker cell (Johansson and Mellander 1975) Excitatory and inhibitory myogenic effector reactions in this spike-generati g type of vascular smooth muscle were shown to be mediated via facilitation and inhibition of normal pacemake generated spontaneous spike discharge Important for the interpretation below was the fact that a strong inhibitory dynamic stimulus caused complete abolition of electrical and hence mechanical activity

These considerations taken together make it quite likely that the myogenic reactions to transmural pressure stimuli in the a te ial microvessels might be mediated via va iations in myogenic pacemake spike discharge as well If so

It would imply that at least a major fraction of the basal tone in the micro vessels is dependent upon action potentials viz that fraction (see Fig 5) which was abolished by the inhibitory dynamic transmural pressure stimulus (dP_T/dt = 5 mm Hg/s) Part of this fraction of basal tone in turn might then be due to a spontaneous pacemaker discharge (like in the portal vein) and the remainder (see Fig 5) to continuous reinforcement of spike activity initiated by the normal arterial pressure via its static and dynamic transmural pressure stimuli

Basal tone in the proximal arterial vessels was little affected by transmural pressure stimuli and its major fraction (Fig 5 white column) might therefore be virtually independent of action potentials Smooth muscle in these vessels might then possibly be of the same non-spike-generating type as present in large arterial vessels (for ref see Johansson 1978) where tone is considered to be caused by contracture and established by graded membrane depolarizations The small fraction of basal tone in the microvessels which remained upon exposure to the strong inhibitory transmural pressure stimulus (Fig 5 white column) might tentatively be explained by a similar mechanism The suggested differentiation of the smooth muscle along the arterial vascular bed in skeletal muscle should not be taken as a distinct feature considerable overlap might well exist

The cause of the common empirical observation that myogenic reactivity can be severely depressed under experimental conditions involving trauma was investigated in paper V It was revealed that this phenomenon which has led to serious questioning of the myogenic hypothesis to a great extent could be explained by stress induced release of catecholamines from the adrenal medulla which via a β adrenergic effect evoked longlasting inhibition of myogenic reactivity during experimental intervention

This observation led to a more detailed study (paper VI) of the interaction between adrenergic and myogenic regulatory mechanisms in an attempt to reveal whether the β-adrenergic vascular control system might serve the function to modify myogenic reactivity also under normal physiological conditions The conclusion was d awn that the sympatho-adrenal system via its neural and humoral β-adrenergic li k exerts a attenuating action on myogenic excitatory reactions which seems to be physiologically meaningful

The finding in this study that the dynamic myogenic microvascular resistance response to a standardized P_T stimulus was of roughly the same magnitude in the

control period as during sympathetic nerve stimulation in the vascular bed with intact adrenoceptors but significantly and gradually increased during nerve excitation after β adrenergic blockade (Fig 6) deserves special attention

It has been clearly established e g by Myers and Honig (1969) that the magnitude of the constrictor response in the resistance vessels to a given excitatory stimulus is increased with increasing level of vascular tone prior to the stimulus This reinforcing phenomenon must be a factor that significantly adds to the complexity in overall vascular control, implying non linearity in peripheral blood flow and resistance regulation This effect as demonstrated in Fig 6 on the β blocked vascular bed was subjected to a specific investigation with the use of the above-mentioned mathematical model (Borgström and Grände 1979; and unpublished data) It was concluded from these analyses that the reinforcing phenomenon mainly could be explained by an increase in gain implicit in the inverse fourth power relationship between microvascular resistance and internal radius according to Poiseuille s law though counteracted to some extent by the concomitant effects of a shift along the smooth muscle length tension curve and of the effect of Laplace law for thick-walled vessels

This reinforcing effect points out some of the complexiti s in peripheral circulatory control where regulatory mechanisms act via influences on the circumferentially oriented smooth muscle cells in the blood vessels As regards the myogenic control system it would seem reasonable that its primary stimulus *in all likelihood the transmural pressure change should* result in a net effector response (e g blood flow or resistance change) which is directly proportional to the size of the primary stimulus irrespective of the prevailing level of vascul r tone Yet the radius factor in Poiseuille s law per se inevitably leads to vast deviation from linearity in circulatory resistance control *even if partially compensated for by negative feedback* inherent in effects of Laplace law nd of possible local accumulation of vasodilator metabolites as blood flow decreases These latter effects however do not suffice to make the magnitude of the myogenic response independent of the prevailing vascular tone (see Fig 6 β blocked vascular bed) but only p ovides coa se djustment It seems that the β adren rgic mechanism in integ ated sympathetic and myogenic control could serve the important function to create fi e adj stment hence making the myogenic control system vi tually linear This conclusion wa derived from the observation on the vascular bed with intact adrenoceptors (Fig 6 lower curve) of an almost equally large dynamic myogenic resistance response to a gi en primary transmural pressure stimulus irrespective of the prevailing level of α adre ergic vascular tone

SUMMARY

This study describes in some detail the characteristics of the myogenic con trol system and of myogenic basal tone in the vascular bed of cat skeletal muscle as well as a pattern of interaction between myogenic and adrenergic me chanisms in circulatory control

Myogenic reactivity was studied in response to graded change of vascular trans mural pressure (P_T) both with regard to its amplitude (static stimulus) and its rate dP_T/dt (dynamic stimulus) With the aid of a specially designed pressure gradient flowmeter and a vascular resistance meter continuous recordings of vascular resistance in the whole vascular bed and in its longitudinal segments the proximal arterial vessels ($>$about 25 µm i d) the microvessels ($<$about 25 µm) and the large veins were obtained The following main con clusions were drawn

1 Myogenic vascular control in skeletal muscle is characterized by a promi nent dynamic or rate-sensitive component developing during phasic change of P_T in addition to the previously known static component developing during constant increased P_T

2 Rate-sensitivity in myogenic control was present only in the microves sels and was bi-directional causing graded excitatory effects (constric tion) in response to positive and graded inhibitory effects (dilation) in response to negative values of dP_T/dt Maximum responses were evoked at positive and negative dP_T/dt values of about 4 mm Hg/s

3 Static myogenic reactivity was present in the proximal arterial vessels and the microvessels whereas the large veins seemed to lack myogenic reactivity entirely

4 A maximal dynamic P_T stimulus was 10 to 15 times more effective in increa sing microvascular resistance than a maximal static stimulus

5 Basal myogenic tone was pronounced in the proximal arterial vessels and the microvessels but almost egligible in the large veins

6 The initiation and maintenance of basal myogenic tone in the microves sels seemed to be intimately related to the arterial blood pressure both via its static mean pressure distension effect and its dynamic pulse pressure distension effect Some 70 % of the normal microvascular tone could be attributed to these stimuli both being about equally effective

7 Basal tone in the microvessels could be almost completely abolished by a maximal inhibitory dynamic P_T stimulus and it was very sensitive to me tabolic stimuli Basal tone in the proximal arterial vessels was vir tually irresponsive to dynamic transmural pressure stimuli and much less sensitive to local metabolic influence

8 *The data indicate that basal myogenic tone in microvessels and proxi-* mal arterial vessels is of somewhat different nature Smooth muscle in the former is tentatively suggested to be mainly of the spike-generating type in the latter it might possibly be of the non spike-generating type

9 In integrated myogenic and adrenergic vascular control myogenic reacti vity seemed to be attenuated by the neural and humoral β-adrenergic mecha nism in a manner which appeared to be physiologically meaningful

10 Falling myogenic reactivity commonly observed during rough expe imental intervention could to a significant extent be expl ined by a β adrenergic inhibitory effect of stress ind ced catecholamine release

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ACTA PHYSIOLOGICA SCANDINAVICA
SUPPLEMENTUM 477

ADAPTIVE CHANGES IN THE SYMPATHETIC NERVOUS SYSTEM AND SOME EFFECTOR ORGANS OF THE RAT FOLLOWING LONG TERM EXERCISE OR COLD ACCLIMATION AND THE ROLE OF CARDIAC SYMPATHETIC NERVES IN THE GENESIS OF COMPENSATORY CARDIAC HYPERTROPHY

By

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CONTENTS

Abbreviations used

ACTH	adrenocorticotropic hormone
BW	body weight
CA	catecholamine
DA	dopamine
ED_{50}	the dose that elicits 50% of the maximum response in the effector cell
M_{50}	the dose that elicits 50% of the maximal pressure response in the perfused organ
NA	noradrenaline
6–OHDA	6–hydroxydopamine
PNMT	phenylethanolamine N–methyltransferase
RNA	ribonucleic acid
S.D.	standard deviation
S.E.	standard error of the mean
SIF cell	Small Intensely Fluorescent cell
VMA	vanillyl mandelic acid
f	degrees of freedom
k	rate constant
	number of observations
n.s.	not significant
$t_{1/2}$	half-life
	versus

Chapter 1

HISTORICAL INTRODUCTION

EXERCISE AND ORGAN SIZE

In 1748 Robinson in his dissertation on the subject of comparative anatomy pointed out that species with a normally high degree of physical activity had a larger heart weight in relation to their body weight than was found in those species whose natural habits involved a lower degree of physical activity. His observations were confirmed and extended by Bergmann, Bollinger and Bauer and Parrot in the late 19th century (quoted by Kulbs, 1906). Furthermore it was observed that within a species individual animals that were subjected to heavy physical exertion, for example race horses and draught dogs (Bollinger 1886) and draught oxen and hounds (Friedberger and Frohner 1896 quoted by Kulbs, 1906) showed an increased heart size. In man Henschen (1898) commented on cardiac hypertrophy occurring in cross-country skiers and Schieffer (1907) showed that regular bicycle riding for three years leads to an increase in heart size.

The first controlled experimental study of the effect of regular physical exercise on the heart seems to have been performed by Kulbs (1906) who exercised two young dogs on a kind of a treadmill for 3–6 months with a final daily exercise load of 3 hours per day. When he compared the hearts of these young dogs with matched control animals whose physical activity had been restricted, the trained dogs were found to have a 52–53% increase in heart weight. Kulbs also later observed that the older the animal the smaller is the degree of cardiac hypertrophy obtained (Kulbs 1912), an observation that has recently been confirmed in the rat by Bloor and Leon (1970). Based on studies on the heart size of wild rabbits, trapped and subsequently kept under conditions that restricted physical activity, Kulbs also stated that exercise induced cardiac hypertrophy can regress (Kulbs, 1912) and this has subsequently been confirmed for rats by Hort (1951) and Leon and Bloor (1968). Likewise Jundell (1912 quoted by Bacher 1923) suggested that the cardiac hypertrophy seen in athletes normally regresses when regular training is abandoned and this was documented in a longitudinal study on a cross-country skier by Deutschen (1920).

Experimental studies have established that regular physical exercise causes cardiac hypertrophy in a number of species such as the dog (see Kulbs 1912, Steinhaus *et al.* 1932), the pig (Kulbs and Berberich 1908 quoted by Kulbs, 1912), the rat (Hasal 1915, Bacher 1921 and a number of later authors) and the rabbit (Hirametsu 1919).

Already in 1912 Kulbs found that there was no great change in the proportion of the total heart weight constituted by each individual heart chamber in dog hearts that had become hypertrophied following regular physical exercise. This was confirmed for the exercise-induced cardiac hypertrophy of the rat heart by Hort (1951) and Van Liere *et al.* (1965).

However, the heart is not the only organ that shows adaptive changes following regular exercise: skeletal muscle mass is increased (Morpurgo 1897, Kulbs, 1912, Petow and Siebert, 1925, Hort, 1951) and the amount of subcutaneous fat is decreased (Kulbs, 1906, 1912, Stevenson *et al.* 1966, Oscai and Holloszy 1969). With moderately heavy physical exercise body weight may remain unchanged (Kulbs, 1906, Hata 1915, Petow and Siebert, 1925) but with fairly intensive exercise the body weight of the trained animals becomes lower than that of control animals (Bezák and Sarkady 1934, Mayer *et al.* 1954, Whitehorn and Grimminger, 1956, Van Liere and Northup 1957, Grimm *et al.* 1963, Leon and Bloor 1968, Oscai and Holloszy 1969).

Hatai (1915) noted that there was an increase in the weight of the adrenal glands in his spontaneously running female rats whereas the increase in the male rats was not significant. Subsequently the weight of the adrenal glands of rats has been found to be increased by exercise in rats of both sexes (Beznak and Sarkady 1934; Hort, 1951; Frenkl and Csalay 1962 Buuck and Tharp, 1971; and later authors).

The weight increase of the adrenals can be accounted for by the increase in size of the adrenal cortex (Beznák and Sarkady 1934 Buuck and Tharp 1971) and this in turn is largely due to increase in width of the zona fasciculata (Hort, 1951 Buuck and Tharp 1971)

The occurrence of adrenal cortical hypertrophy is not surprising as it has been shown that physical exertion leads to secretion of adrenocortical hormones. This was first suggested by the decrease in cholesterol content of the adrenal cortex following exercise described by de Jongh and Rosenthal (1933) subsequently physical exertio was found to lead to the excretion in the urine of increased amounts of adrenocortical hormones (Venning and Kazmin 1946) and finally it was shown that blood levels of corticosteroids in the rat particularly corticosterone were increased by exercise in man (Froesch *et al.*, 1954 and later authors) in dogs (Nazar 1971) as well as in rats (Frenkl *et al* 1968 1969 Buuck and Tharp, 1971; for extensive references see Nazar 1971).

The increase in size of the adrenal glands occurs within two weeks of the start of regular exercise (Beznak and Perjés, 1935 Hort, 1951 Frenkl and Csalay 1962) and the size of these glands begins to regress upon cessation of exercise (Beznak and Perjés, 1935 Hort, 1951)

The influence of regular exercise on the size of the spleen has been the subject of conflicting reports. Thus Kuibs (1906 1912) found no consistent change in the dog as did Hort (1951) in the rat, whereas Hatai (1915) found a smaller spleen in his exercised rats. Petow and Siebert (1926), also using rats found a lower splenic weight only in the group that ran slowly for 320 min/day not in the group that ran at a higher speed for 20 min/day although the latter group had the more pronounced degree of cardiac hypertrophy

THE INFLUENCE OF ACUTE EXERTION ON THE SYMPATHO–ADRENAL SYSTEM

The first observations on the role of the sympatho-adrenal system i e ercise appears to have been the demonstration by bio-assay on the denervated iris by Hartmann *et al* (1922) of the release of adrenaline into the blood during muscular work and this observation was conf rmed on the denervated heart by Cannon *et al* (1924) Wada *et al* (1935) studied the secretion of adrenomedullary hormones in the venous effluent from an exteriorized adrenal gland in conscious dogs subjected to exercise and found that only xhaustive work, but not running o moderate intensity lead to significant increase in secretion of medullary hormone That muscular work leads to an increased excretion of noradrenaline (NA) in the urine was first shown by bio-assay by Holtz *et al* (1947)

It has been concluded that adrenaline present in the urine mainly originates from the adrenal medulla whereas the NA excreted in the urine largely is derived from sympathetic nerve-endings (Euler *et al* 1954) *The first study that related the urinary xcretion of both NA and adrenaline to the intensity of* xercise was performed by Euler and Hellner (1952). They found in men that exercise f moderate intensity with an oxygen consumption not xceeding 2 l/min caused only a small increase in the amount of adrenaline excreted in the urine whereas at high work loads the xcretion f adrenaline in the urine was increased 5–20 fold Likewise excretion of NA in the urine was modestly increased at moderate work rates whereas at high work loads the urinary excretion of NA was increased up to 10 fold.

Vendsalu (1960) found that in venous plasma the increases in absolute concentrations of catecholamine (CA) with increasing work-load were smaller for adrenaline than for NA, particularly at high work-loads. However if the increases are expressed in relation to the resting concentrations then his data actually show that in males at work-load of 600 kpm, the plasma concentration of adrenaline is 3-fold greater than at rest whereas that of NA is only 2-fold greater at a higher work-load of 1200 kpm the concentrations of both adrenaline and NA were 5.8-fold higher than at rest. A similar trend was seen in the females studied by Vendsalu. Thus it appears that, relative to rest, there is greater change in the adrenaline concentration than in the NA concentration at low work-loads. It is striking that at high work-loads there was no change in the relative proportion of adrenaline to NA in plasma compared with the proportion in plasma taken at rest. Furthermore data has been presented by Häggendal *et al* (1970) suggesting that NA levels in arterial plasma increase exponentially with increasing work load and are similar in different individuals if the relative work load is the same

The difference in the degree of activation of the sympathetic nervous system and the adrenal medulla has been attributed to their different actions, the sympathetic nervous activity being involved in the cardiovascular readjustments to exercise and the prevention of hypotension resulting from regional vasodilatation whereas the excretion of adrenaline from the adrenal medulla is thought to be mainly triggered by the accompanying elements of emotional stress and by exercise-induced hypoglycaemia (see reviews by Euler 1974 and Frankenhaeuser 1971).

During exercise the increase in cardiac output, necessary to supply the increased amounts of oxygen required by the working muscles, is achieved mainly by increasing the heart rate whereas the stroke volume during supine exercise only increases by about 10—20% (see review by Bevegard and Shepherd, 1967) Although the Frank—Starling mechanism contributes to the beat to beat regulation f cardiac output the large alterations in cardiac output induced by exercise are therefore achieved by the autonomic nervous system

The increase in heart rate occurring during exercise can, in normal heart, be assumed to be due largely to increased activity in cardiac sympathetic nerves, rather than to increased concentrations of circulating catecholamines or to decreased vagal tone for the following reasons. (i) There is normally a simultaneous increase in myocardial contractility which would not be expected to occur after simple vagal inhibition (ii) vagal blockade alone does not achieve maximal heart rate and does not increase cardiac output, furthermore the mechanical systole is longer and stroke volume is smaller than when the same heart rate is achieved by exercise. Also the maximal heart rate achieved through exercise is not influenced by atropine (see review by Bevegard & Shepherd, 1967). (iii) Although both circulating NA and adrenaline have positive inotropic and chronotropic actions, the experiments of Donald and Shepherd (1964) on greyhounds with surgically denervated hearts demonstrated that the increase in cardiac output achieved in the denervated heart by circulating catecholamines was of slower onset than that seen in intact dogs and was qualitatively different, with smaller increase in heart rate and a larger increase in stroke volume from the changes found in unoperated dogs at the same cardiac output (Donald and Shepherd 1964).

Evidence consistent with increased sympathetic nervous activity in the heart of rats during exercise was produced by Gordon *et al* (1966[b]) who found that after inhibition f NA synthesis with α-methyl para-tyrosine the NA levels of the heart fell significantly more rapidly in rats subjected to exercise than in sedentary rats, implying greater rate of turnover of NA in the xercised animals.

MODIFICATIONS IN THE SYMPATHO–ADRENAL SYSTEM INDUCED BY PHYSICAL TRAINING

Euler and Hellner (1952) in their study on urinary excretio f catecholamines in men following xercise observed that one of their subjects an athlete considerably bette trained than the other subjects showed in contrast to the other individuals no increase in the amount of NA excreted in the urine at work load intensities up to an oxygen uptake of 2.1 l/min The role of physical fitness in the activation of the sympatho-adrenal system during muscle work remained controversial however since Vendsal (1960) did not find any significant d fference in the plasma levels of NA in physically fit group compared with less fit subjects working at the same work load whil on the other hand Carlsson *et al* (1968) in a study on mentally disordered patients, found that the plasma NA levels at a given work load were lower in patients who had been subjected to physical training.

It has long been established that athletes display phenomenon called relative bradycardia with a slower heart rate and larger stroke volume both at rest and during any given work load and as the ma imum heart rate is the same they also have highe maximum cardiac output (Dedichen 1920 Herxheimer 1921 Kauf 1928 Bock *et al* 1928 Hoogerwerf 1929 Schneider and Crampton, 1940 and later authors see review by Bevegard nd Shepherd 1967) The relative bradycardia can develop after a fairly limited period of vigorous training both in humans (Knehr *et al* 1942) and n an mals such as the rat (Hort, 1951 Tipton 1965 Buuck and Tharp 1971) The bradycardia develops roughly in parallel with the development of cardiac hypertrophy (Tipton 1965 Buuck and Tharp 1971) Since stimulation f the vagus nerve causes cardiac slowing, an obvious hypothetical explanation of the relative bradycardia f the athletic heart was that athletes developed an increased vagal tone at least in the efferent fibers to the heart This hypothesis has become very popular (Kauf 1926 Steinhaus, 1933 Mellerowicz 1956 Herrlich *et al* 1960) However the xperimental evidence is not at all convincing Herxheimer (1921) quotes several studies showing that 1 mg f atropine sulphate causes an increase in heart rate f between 20–60 beats/min in the general populatio and then report his study on thletes where this dose in spite f causing dryness f the mouth lead only to a mi imal acceleration f heart rate about 5 beats/mi and in some individual had no effect on heart rate at all He concluded that since the bradycardia of the athlet heart could not be abolished by atropine it was not due to increased vagal tone Likewise Kauf (1926) found no cardiac acceleration in athletes after administration f 1 mg of atropine sulphate but he interpreted the lack of ffect of atropine to be due to massive vagal tone that could not be overcome by usual doses, and he was able to show that after 2 mg of atropine there was some cardiac acceleration in athletes although only about 70% of the heart rat increase seen in his control group fter half the dose

Since atropine sulphate crosses the blood-br in barrier is possible that the higher dose of atropine used by Kauf erted its ffect through actions on the central nervous system The latt possibility is supported by the finding of Robinson *et al* (1953) hat 2 mg f atropine sulphate caused restlessness in the xperimental subjects and lead to an increase in the O_2–consumption at rest Furthermore there are several observations suggesting that increased vagal tone alone cannot xplain the bradycardia in athletes. Firstly as discussed by Reindell *et al* in he eview (1954) the athletic heart classically displays both prolonged isomet ic period f the systole nd prolonged total syst le and vagal stimulation or acetylcholine administration cannot herefore alone simulat the cardiovascular characteristics of athletes Secondly Raab *et al* (1960) were not able to abolish the d fference in mean heart ate between a physically active group f men and relatively inactive control group by fairly large doses of atropine sulphate (0.025 mg/kg) around 1.75 mg/person Thirdly Tipton (1965) showed hat

relative bradycardia develops in response to training in rats that have had their right or left vagus cut, and that atropine causes less cardiac acceleration in trained than in untrained rats (Tipton and Taylor 1965)

Herrlich *et al* (1960) base their proposal f excessive vagal tone on findings of increased acetylcholine and/or acetyl cholinesterase levels in the heart but, as will also be illustrated in Sections C and F (Chapter 3) on the NA stores of the heart, organ transmitter concentration does not always parallel transmitter turnover and thus these findings do not in themselves indicate an increased parasympathetic tone

An alternative explanation for the bradycardia of the athlete s heart would be that rather than an increase in vagal tone there was instead decrease in nervous activity in the sympathetic nerves innervating the heart. In study specifically designed to look at the role of cardiac sympathetic nerves in the bradycardia of the athletic heart De Schryver *et al* (1967) claimed that the reduction f heart rate was due to depletion of NA however th training intensity used in this study was too low (see Section B, Chapter 3 for further discussion) to produce the usual criteria for successful training effect such as cardiac hypertrophy and reduction in body weight. In summary therefore as concluded also by Åstrand and Rodahl (1970) the origin of the relative bradycardia in the athlete remains obscure.

AIMS OF THE PRESENT STUDY

In 1932 Cannon wrote: "The man who daily takes cold bath and works until he sweats may be keeping "fit because he is not permitting very valuable part of his bodily organization (the sympatho-adrenal system) to become weakened and inefficient by disuse

However although there has been lot of experimental research studying the changes in activity f the sympatho-adrenal system during various forms of physical and emotional stress, in 1969 comparatively little was known about the effects of chronic alterations in the activity f the sympatho-adrenal system. Chro ic exposure to cold had been shown to lead to acclimation involving among ther things increased sensitivity to calorigenic effects of NA, lower excretion of NA in the urine during cold exposure and larger capacity to survive cold xposure (refs see Introduction to Chapter 4) Chronic exposure to "emotional stress had been studied by Welch and Welch in spontaneously fighting mice and found to lead to increases in NA content in brain and heart and increases in adrenal adrenaline content (Welch, 1967 Welch and Welch, 1969) However there did not appear to have been any studies on the effect of chronic physical stress on the activity of sympathetic nerves in various organs, and of the secretory activity f the adrenal medulla when the animal was at rest and undisturbed.

The main aims f the present thesis were therefore to study the following questions:

1 Does the sympatho-adrenal system adapt to chronic increase in its activity?

2. If so — how is this adaptation xpressed?

3 How does adaptation to chronic xercise compare with acclimation to cold?

4 What is the role of cardiac sympathetic nerves in the relative bradycardia of the athletic heart?

5 What is the role of the cardiac sympathetic nerves in the development of exercise-induced cardiac hypertrophy?

Chapter 2

GENERAL METHODS

EXPERIMENTAL ANIMALS

The animals used in most of the experiments were male albino rats of the Sprague—Dawley strain, obtained from Anticimex AB, Stockholm. In one experiment, male Wistar rats from Charles River Ltd United Kingdom, were used. The rats were kept at room temperature unless otherwise stated, and had free access to water and food (standard chow for rats). They were kept 4 to 6 rats (depending on age and size) to a cage of 56 by 37 cm.

TRAINING METHODS

Running

The experiments where the animals were trained by running were performed using a motor-driven treadmill. As the rats would not run spontaneously throughout the whole training session and some rats developed a very expert technique in effortlessly sliding along comfortably resting against the back wall of the treadmill, it was necessary to discourage the rat from touching the back wall. This was achieved with the help of an electric fence on the back wall of the treadmill which intermittently delivered weak electric shocks. Following such negative inducement the rats quickly learned to keep running at a steady pace to avoid contact with the back fence. After about 4 weeks adaptation to the treadmill actual training was started at a speed of 22 m/min for 1 h/day and the duration and intensity of the exercise session were gradually increased, as described in detail in paper I, so that during the last 6 weeks the rats ran at 37 m/min for two 40 min sessions with a 10 min period of rest in between, covering a total distance of 2,640 m/day.

General comments on treadmill training. The inbred albino rat is not a very good runner for its size. Furthermore, the occasional weak electric shock received by the poor runners adds an undesirable element of "emotional" stress to a study on exercise. Towards the end of the study, when the exercise load was high, it was occasionally noted that a shock made the receiving rat so aggressive as to start a fight with another rat in the treadmill; this was however by no means a regular feature.

Swimming

Since treadmill running did not seem to be a very satisfactory method for the regular long term exercise of rats, it was decided instead to try regular swimming as an alternative exercise method.

The water temperature was kept at 35 ± 1 C. Most authors using swimming as a means of exercise use water temperatures between 33 C—35 C (Crews and Aldinger, 1967; Lin and Horvath, 1972; Leon *et al.*, 1975) and Lin and Horvath (1972) state that rats maintain a normal body temperature during swimming at 35 C; furthermore Tan *et al.* (1954) showed that a water temperature of about 36 C is optimal for an untrained rat, the maximal swimming time getting shorter at temperatures both above and below. The fact that a water temperature slightly below body temperature is optimal is probably because it allows the animal to get rid of the excess heat produced by muscular work without actually becoming hyperthermic.

When rat is put into water to swim for the very first time it appears at first surprised and usually just floats on the air trapped in the fur for moment, following which it rapidly discovers how to swim about by propelling itself with its feet. Subsequently it swims rapidly around the edges of the bath for couple of minutes apparently searching for a way t climb out. When this search has proven futile an untrained rat then settles for the minimum level of xertion necessary "treading water" to keep afloat

Before training was started with long daily sessions, the rats in the training group were accustomed to water over a period f about five days the first day each rat was left only 5 min in the water the second day 15 min and thereafter increasing by 15 min/day until daily session of 1 h/day was reached. After 4 weeks of training at this load the daily swimming periods were stepwise increased by 30 min at time until final daily exercise period of 2 h or in papers IV and V 2,5 h was reached. In the swimming baths measuring (length width height) 70 X 44 X 53 cm, 8–10 rats swam together in each bath (paper II) in the baths measuring 100 X 70 X 60 cm (papers III–VI) 12–15 rats swam simultaneously

Following each swimming period the rats were transferred to "drying cage" containing several layers f soft blotting paper where they immediately proceeded to shake off the water and started drying their fur without showing any signs of excessive fatigue. After about 20 min, their fur was virtually dry and they were transferred back to their ordinary cages where they immediately started eating, in contrast to our rats trained by treadmill running which never eat shortly after the exercise presumably due to running-induced hyperthermia I the studies on NA turnover and urinary excretion of catecholamines during and after exercise in papers II III and VI, the controls and trained rats swam together in the same bath during the acute exercise session. I this way the swimming activity becomes subjectively uniform in the two groups.

General comments on swim training Having experience of both methods of training rats described above it is my firm opinion that, perhaps surprisingly swim training is the method which involves the least "emotional stress. The initial alarm reaction in the rat when left in the water as described above soon disappears as the rat gets accustomed to the water and the fact that it will invariably be picked up again Subsequently the rats merely start swimming about at a fairly leisurely pace and sometimes spontaneously dive and swim about under water to xplore the bottom f the tank.

Rats practically never aspirate water during training sessions as also indicated by the fact that there was no morbidity whatsoever in chest infections (which might have been xpected t occur from aspiration of the bath water that was always somewhat contaminated by fecal material), furthermore among the total of 179 rats trained by daily swimming in the various experiments there was not a single fatality due to drowning.

Cold-exposure experiments

Cold acclimation was achieved by keeping rats for 14 weeks at constant temperature of 4 C ± 1 C, in plastic cages furnished by shavings with 4–6 rats per cage and with food and water *ad libitum*. Illumination essentially followed normal office hours. During acute cold xposure rats were kept, for 22 h at 4 C, singly without any bedding, in metabolic cages for urine collection and with free access to food and water

Activity studies

Spontaneous physical activity was measured by a photoelectric (Motron) activity meter with a photo cell floor under fairly intensive illumination during daytime.

Preparations of organs and tissue extracts

Rats were killed by exsanguination under light ether anaesthesia. Organs were quickly dissected out and carefully trimmed and the heart cut open to expose both ventricles and carefully blotted before weighing. Brains, hearts, spleens and submandibular glands were homogenized with an Ultra-Turrax apparatus in 15 ml ice-cold 0.4 M perchloric acid containing 0.1 mM ascorbic acid. The homogenates were centrifuged to sediment the insoluble matter and in most studies the supernatant was titrated to pH 3.8 with KOH and recentrifuged for the elimination of potassium perchlorate and the supernatant was kept frozen at −20 C until the assay was performed. Catecholamines were adsorbed on alumina, eluted and oxidized according to Chang (1964) or according to Euler and Lishajko (1961) as described in detail by Swedin (1971). In the turnover study using α-methylparatyrosine (Chapter 5) it was necessary to use the Chang (1964) method, since the Euler–Lishajko (1961) method gave falsely high results.

The recovery figures for NA added to random samples and carried through the extraction and oxidation procedures were 84.4 ± 3.8% in heart homogenates, 89.3 ± 2.3% in spleen homogenates and 77.1 ± 5.1% in submandibular gland homogenates. Data given in the papers are uncorrected for recovery and expressed as μg free base. Extracts from adrenal glands were oxidized according to Euler and Lishajko (1961) or Chang (1964) directly without prior adsorption to alumina. In the study published in paper V the supernatant of perchloric acid organ homogenates was adsorbed on alumina columns, from the effluent tyrosine was isolated on Amberlite CG 120 columns and in the eluate from the alumina column NA and dopamine (DA) were separated using Dowex 50W–X4 columns; all procedures were as described in detail by Nybäck (1971). DA was assayed according to the method described by Carlsson and Waldeck (1958) and Carlsson and Lindqvist (1962) and NA according to Chang (1964). The tyrosine content in the Amberlite eluate was determined according to Wong *et al.* (1964). In paper V the recovery of exogenous radioactive NA and DA added to brain extracts before the passage over the various columns as described was 50% for NA and 42% for DA.

Urine collection and assay of urinary catecholamines and VMA. Urine was collected in metabolic cages where the animals were kept individually over a period of 22 h. Contamination of collected urine by food and fecal particles were minimized in paper II by the use of a glass drop-ball below the funnel and in paper III and VI by using glass fibre wool as a filter in the funnel. When urine collection was performed after a swimming session the rat was lifted out of the bath, manually blotted with blotting paper and an attempt was made to initiate reflex emptying of the urine bladder by suprapubic pressure. The latter procedure was never successful possibly because the bladder was already empty. Rats do defecate in the water while swimming and may well urinate then too. The collection bottles contained, at the start, enough 1M HCl (4–6 ml) to keep the final pH to 4 or below. After removal of the rats, the funnels of the metabolism cages were rinsed with 10 ml of 0.1M HCl and the rinsing fluid that filtered through was included in the sample. The pH values of all urine samples were checked and virtually all samples had a pH considerably below 4.0; the results of those samples where the pH exceeded 4 (usually due to heavy leakage of the water bottle in the cage) were discarded. Urine was centrifuged

for 15 min at 9000 r.p.m., the supernatant was titrated to pH 3.8 with 2M KOH and recentrifuged and the supernatant kept frozen at −20 C until the assay was performed.

For the assay of catecholamines one tenth volume of 0.27 M disodium EDTA was added to the urine sample which then passed through an alumina column that was eluted as described by Swedin (1971). The NA and adrenaline content was assayed according to Euler and Lishajko (1961) or Cheng (1964) and is expressed as μg free base.

Vanillylmandelic acid (VMA) was estimated by the method of Pisano *et al.* (1962) which involved oxidation with sodium periodate. 7 ml of urine was shaken with ca. 0.5 g of Florisil and the VMA in 5 ml of the supernatant was extracted and oxidized as described by Pisano *et al.* (1962). Excess periodate was removed by adding glycerol. The reaction product was extracted into toluene and the optical density at 360 mμ was measured. Because of variation between the amount of contaminating material present, a blank tube for each sample of urine was prepared in which an excess of glycerol was added before the periodate. Internal standards, containing 10 μg of VMA, were done for each set of oxidations.

Sympathetic ganglia were homogenized in 0.3 M sucrose and tyrosine hydroxylase activity was assayed according to Nagatsu *et al.* (1964) using 3,5−(H)−L−tyrosine.

Isolated organs

Isolated heart. Half an hour before the experiment the rat was pretreated with 800 I.U. heparin intraperitoneally. The animal was anaesthetized with an intraperitoneal injection of sodium pentobarbitone (Mebumal sodium) 5 mg/100 g B.W. The beating heart was excised with one cut and immediately transferred to ice-cold 0.9% (w/v) NaCl which stopped the heart beating within a few seconds. After a quick rinse in saline the cut end of the aorta was identified and any large bulk of extraneous tissue excised. The aorta was cannulated and retrograde perfusion of the heart by the Langendorff procedure was commenced, following which the heart resumed beating almost immediately. At the same time 5 ml of heparin solution 10 mg/ml dissolved in the perfusion medium, was slowly injected in the perfusion cannula. Any remaining extraneous tissue was trimmed away and free outflow from pulmonary artery was secured.

The perfusion medium was a Krebs−Henseleit bicarbonate buffer (118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$) with the addition of 14 mM glucose, 0.5 mM of disodium ethylenediamine tetra-acetate (EDTA) titrated to pH 7.40 and 0.11 mM ascorbic acid. The perfusion medium was oxygenated by a gas mixture of 95% O_2 and 5% CO_2 and had been equilibrated with the O_2 CO_2 mixture at room temperature for at least 20 min before the perfusion was commenced. The pH of the equilibrated medium with additions was 7.40. The heart was perfused by the perfusion fluid at 38 C (higher temperatures increased the liability to arrhythmias) and was surrounded by a water jacket maintained at 38 C, with the top sealed by Parafilm. The perfusion fluid was delivered to the heart at constant rate via a peristaltic pump and there was no recycling of used Krebs solution. As the hearts of trained rats on the average are about 7% heavier than those of control rats it was necessary to have a correspondingly higher perfusion rate for the hearts of trained rats, in order to achieve the same average perfusion rate in flow per g heart. The ideal flow rate was considered to be that which gave an average initial perfusion pressure of about 60 mm Hg in both experimental groups, as 60 mm Hg was considered by Neely *et al.* (1967) to be the optimal perfusion pressure for isolated hearts. In pilot experiments a flow rate of 17.2 ml/min was found to be

suitable for control hearts and a flow rate 18.5 ml/min for hearts of trained rats, which resulted in an average flow of 13.4 ml/min/g heart in both experimental groups. The perfused hearts were weighed after perfusion and it was found that the average heart weight of the two groups did not differ significantly from the expected ratio as the mean heart weight in the trained group was 8% higher than that in the control group. The perfusion pressure at the start of the determination of dose response curves averaged 68 ± 7 mm Hg in the control group and 70 ± 8 mm Hg in the hearts from trained rats. The perfusion pressure usually rose somewhat during the experiment averaging 85 mm Hg in both experimental groups at the end of the experiment

Left ventricular contractions were recorded with a rubber balloon inserted via the mitral valve and inflated *in situ* with a pressure of 5 cm H_2O. The intra balloon pressure was recorded via Statham pressure transducer

Initially I attempted to obtain a dose-response curve via infusion of NA at a constant rate by an automatic syringe maintaining each infusion rate until a steady state heart rate was obtained. This however proved to be impossible in practice since it induced a very high rate of arrhythmias. It was therefore necessary to give each dose as 10 second period of infusion of NA at constant concentration and to allow the heart to recover between each dose The dose given in the dose response curves is that infused during the 10 seconds.

During each experiment the temperature of the perfusion fluid the perfusion pressure the left ventricular balloon pressure and the heart rate was continuously recorded on Grass Polygraph Recorder and the rate of perfusion was monitored by measuring the effluent from the heart.

Perfused hindbody The perfused hindbody of the rat was prepared as described by Folkow *et al* (1970) with only minor modifications. Rats were treated with 500 I U heparin i.p. 30 min before the experiment and anaesthetized with 6 mg/100 g BW of sodium pentobarbitone (Mebumal). The rat was eviscerated, with care being taken to tie off all severed vessels, and the aorta was cannulated above the iliac arborizations as described by Folkow *et al* (1970) The vena cava was opened wide to provide free outflow. After the hindbody had been isolated from the upper part with standardized mass ligatures it was cut away from the upper half and the hindfeet and tail were tied off to minimize cutaneous circulation The isolated hindbody was placed in plastic chamber with drain for the outflow heated by a 38°C water jacket and covered by plastic film to keep temperature and humidity constant. The hindbody was perfused by Krebs—Henseleit's buffer (the same as used for the isolated heart) with the addition of 5.5 mM of glucose 0.11 mM ascorbic acid and 4% (w/v) of Ficoll (a synthetic colloid a polymer of sucrose and epichlorohydrin) with average molecular weight of 77 000 kindly supplied by Pharmacia Fine Chemicals. In second series 2 µm disodium EDTA was added as well. The perfusion medium was oxygenated with 95% O_2 5% CO_2 gas mixture and when equilibrated the pH of the medium including additives was 7 40 The hindbodies of both groups were perfused at the same rate averaging between 10—11 ml of perfusate/100 g tissue each minute and the perfusion fluid was kept at 38 C. However unlike Folkow *et al* (1970) only one hindbody was perfused at time As described by Folkow *et al* (1970) slug injections of 1—3 mg papaverine was given initially to ensure maximal vasodilatation however there was never any further drop in peripheral resistance following these injections The papaverine was allowed 20 min wash out period before the NA infusion was commenced. The NA was infused using motor driven syringe at constant rate that was increased in a stepwise fashion and the perfusion pressure was continuously recorded via Statham pressure transducer — at the aortic level At high concentrations of NA there was not infrequently small initial 'overshoot' in perfusion pressure before stabilized at slightly lower value in all such instances the steady-state

pressure was used in the calculations of the dose-response curve. Towards the end of the experiment, there was as described by Folkow et al (1970) some tissue oedema despite of the use of colloids, but there was no difference between the two groups in this respect.

DRUGS

l-noradrenaline bitartrate (Sigma chemicals) and dl-isoprenaline sulphate (Abbot Laboratories) were used and concentrations calculated as μM base. DL-4-hydroxy-3-methoxy-mandelic acid (Sigma Chemicals) was used as standard in the VMA assay. For the infusion solutions, high concentrations of noradrenaline was dissolved in 0.9% NaCl with 0.11 mM ascorbic acid and 1.0 mM EDTA in order to obtain maximal stability of the catecholamine. Isoprenaline was dissolved in 0.9% NaCl with 0.11 mM ascorbic acid.

STATISTICS

Significance of difference between the means of various groups were calculated with the help of Student t-test. Half-lives and significances of differences between rate constants were calculated using conventional statistical methods (Edwards, 1954, Goldstein et al 1969).

Chapter 3

EFFECT OF REGULAR PHYSICAL EXERCISE (TRAINING)

SECTION A GENERAL OBSERVATIONS

EXERCISE TOLERANCE AND SWIMMING BEHAVIOUR

As judged by their behaviou untrained rats when swimming 8 rats per bath are moderately fatigued fter 2 h of swimming, fairly xhausted after 4 h f swimming and very exhausted after 8 h of continuous swimming. The trained rats on the ther hand looked very little affected even after 8 h of swimming (paper II) It is clear from simple observation that the greater the number of rats per unit surface area in the bath the greater the swimming activity and therefore it is important for such data to be included in publications describing studies on swim training.

When trained and untrained rats are exercised in equal umbers in separate swimming tanks, it is noticeable that the swimming activity f the trained rats is higher than that of untrained rats as the trained rats spontaneously swim about whereas the untrained rats tend to "tread water" a lot of the time. This d fference in activity appears to be l minated when trained a d untrained rats are put i the same tank in reasonable numbers and such procedure is therefore an essential precaution i any study directly comparing trained and untrained rats during swimming.

Aggressive behaviour during xercise

When under stress male rats frequently start to fight each other This was not nfrequently observed in the rats during forced running in the treadmill toward the end f the longer exercise sessions.

The incidence of stress-induced aggressive behaviour was much lower du ing swim training than during treadmill running it only occurred in one out of four studies the one described in paper IV where the density of rats in the bath was somewhat higher than the others. Even in this study only 3 out f 72 rats displayed an aggressive behaviour and when these rats were isolated a separate bath this behaviour subsided and there were no further problems. These 3 rats were all included in the group studied 6 months after cessatio f exercise

Behaviour after exercise

After a long session f forced running rats salivated profusely drank lot but xhibited decreased spontaneous motor activity and did not eat f at least half an hour I contrast after swim xercise the trained rats immediately proceeded to groom themselves and subsequently started to eat This difference behaviour is probably largely due to the hyperthermia nduced by running, salivation being an important part f thermoregulation in the rat (Hainsworth, 1967 Ekstrom, 1974)

There was subjective impression that the spontaneous motor activity of resting chronically xercised rats was slightly higher than that of control This mpressio was however not verified in studies in illuminated photocell-type activity cages where the spontaneous activity f both groups of ats was found to be the same

SECTION B EFFECT OF CHRONIC EXERCISE ON BODY AND ORGAN WEIGHT

(Papers I II, III V VI)

RESULTS

Body weight

In all studies using albino rats, regardless of the type f exercise used, chronic physical exercise was found to decrease the rate of weight gain during growth without influencing body length, so that at the end of the training period the body weight was from 5 to 8% lower than in controls (papers I II, III, V)

Heart

I all the studies on chronically xercised animals the hearts were found to have hypertrophied. The degree of cardiac hypertrophy was remarkably constant in all studies irrespective of the type of training and variations in duration f training, being 7%, measured as absolute heart weight, in the studies described in papers I II III and V

Adrenal glands

Adrenal hypertrophy was most pronounced in rats trained by forced running, with weight increase of 31% (paper I) but was significant also in swim-trained rats ranging from +8 to +10% (papers II and III)

Spleen

In paper II the spleen weights of chronically xercised rats were found to be on average 8% lower than those f controls but this difference was not significant: in paper V however the 9% decrease in splenic weight i the trained group was statistically significant ($p < 0.01$).

The weights of *brain* and *submandibular glands* remain unaltered in rats fter chronic physical xercise by swimming (paper V III).

6 months after cessation f chronic exercise there is no significant difference between body weight, adrenal weight and splenic weight between -trained rats and controls (paper III and unpublished observations) Although the bsolute heart weight was 5% higher in trained rats this is no longer significantly different from controls, but the heart ratio in trained rats remains very significantly higher than the control value (10%, $p < 0.001$). In this case the fact that the difference in absolute heart weight is no longer significant is due to the increasing individual variation in size with increasing age. This is so because even f the contribution of the non-significant −3% difference in mean body weight between the two groups is allowed for the difference in heart ratio still remains statistically significant.

DISCUSSION

Body weight

That rats subjected to heavy chronic physical exercise gain less weight than control rats was pointed out already by Bazmak and Sarkady (1934) and subsequent authors (see Chapter 1). This effect is not always seen with low-intensity exercise however prolonged (Pitow and Siebert, 1926) and at least in adult rats, is often only seen after daily and not after intermittent exercise (see Section E). The decreased weight gain seen in rats subjected to daily exercise is due to a relative hypophagia on the day of exercise. Mayer *et al.* (1954) using treadmill running found that light exercise did not increase food intake but actually decreased it below control values, whereas with increasingly long daily exercise sessions the rats would increase their food intake but never enough to compensate fully for the increased energy need and consequently the body weights of the trained rats always remained below control values. The depression of food intake is not solely due to hyperthermia induced by muscular exertion since it is also present in rats which are made hypothermic by swimming in water at 30 C (Stevenson *et al.* 1966) although to a lesser degree than in the rats exercised by treadmill running. The resulting decrease in body weight is due to a reduction in white fat tissue which is not fully compensated for by the increase in weight of skeletal muscle occurring in trained rats (references see Chapter 1) and thus the proportion of the body weight constituted by fat decreases in parallel with increasing exercise load (Stevenson *et al.* 1966, Hanson *et al.* 1967). After single bouts of exercise however there is a compensatory increase in food intake during the following two days (Edholm *et al.* 1955, Thomas and Miller 1958, Stevenson *et al.*, 1966); this explains the lack of effect on body weight after intermittent training.

The effect of exercise on body weight appears to be less marked in female than in male rats (Van Liere and Northup 1957). Richter (1968) found that untrained female rats subjected to extremely long periods of swimming (40–60 h) subsequently developed a syndrom characterized by hyperphagia and obesity as well as pseudopregnancy. These observations could explain why Crews and Aldinger (1967) who subjected female rats to stressful daily swimming for 6 h/day found no effect on their body weight.

There is no evidence that the effect of exercise on body weight is due to increased thyroid activity as mentioned above; trained rats display a relative bradycardia and as shown in Section G of this chapter the intrinsic heart rate of isolated hearts is not altered, whereas it is well known that hyperthyroidism causes an increase in the intrinsic heart rate of the isolated heart (see review by Freedberg and Hamolsky 1974).

Heart

That daily physical exertion leads to adaptive growth of the heart has been shown for many species and many different forms of exercise (see Chapter 1 for references). This adaptive hypertrophy does not require the presence of either growth hormone or thyroxine or any other pituitary factor as demonstrated by Tipton and Tcheng (1971), who found that forced running still produced cardiac hypertrophy in hypophysectomized rats. Development of adaptive growth of the heart in response to chronic exercise does however seem to require the presence of cardiac sympathetic nerves since it is abolished after chemical sympathectomy (paper VI, see Chapters 6 and 7 for further discussion).

Exercise-induced cardiac hypertrophy involves equally both the right and left ventricles (references

see Chapter 1) and leads to an increase in both the longitudinal and transverse diameters f the heart (Hort 1951) and thus also in total volume (Crews and Aldinger 1967) Malik et al (1974) who studied the compensatory hypertrophy in dogs after chronic pressure over-load (experimental aortic stenosis) and fter chronic volume over-load (chronic arterio-venous fistula) found that whereas the left ventricular wall thickness was increased in both types f hypertrophy only chronic volume over-load produced an increase in the internal diameter f the left ventricle Since the increase in cardiac work during exercise is mainly due to increased cardiac output, the findings by Malik et al (1974) in the high output state are probably relevant to discussion of exercise-induced changes. The finding that athletes have larger stroke volume than untrained subjects not only during rest but throughout xercise and p to the maximal heart rate (Bock et al 1928 T Sjostrand 1955 Wang et al 1961 Bevegard 1962 Bevegard et al 1963 Astrand et al 1964) could be xplained ither by increased sympathetic nervous activity (causing sh ft in the Frank—Starling relationship) or by an increase in internal ventricular dimensions as result of exercise-induced cardiac hypertrophy Our findings of lower level of sympatho-adrenal activity in trained rats, compared with controls during exercise as well as rest (see Sections D & F) would, if it is also true in man mean that the greater stroke volume of athletes is due to change in the dimensions of the ventricle.

The increase n heart weight seen following chronic physical xercise is not due to an increase i cardiac muscle mass alone as there is an increase of collagen content parallel to that in heart weight (Bartosova et al 1969) Furthermore xercise leads to the formation of new capillaries (Mandache et al 1972 Ljungqvist and Unge, 1972 Mandache et al 1973) with resultant ncrease in capillary/fibre ratio (Thorner 1935 Petrén et al 1936 Petren and Sylven, 1937 Leon and Bloor 1968 Bloor and Leon, 1970 Ljungqvist and Unge 1972) and the dimensions of coronary and extra-coronary anastomosing arteries also increases (Leon and Bloor 1968 Bloor and Leon, 1970) In the rat some xperimental conditions l k altitude (Sizemore et al 1973) carbon monoxide exposure (Penney et al 1974) chronic isoprenaline admi istration (Stanton et al 1969 Le Blanc et al 1972) and xper mental hyperthyroidism (see review by Korecky and Beznák 1971) can lead to massive hypertrophy with increases f 50—70% in the weight of one or both ventricles of the heart in relatively short space f time sometimes only 10 days In man prolonged xposure to pathological conditions can lead to increases of 100% and more in the weight f the heart (Brown 1971) I comparison with these pathological changes, the degree of xercise-induced cardiac hypertrophy in these studies (7 to +8% I papers I—VI and Section E) looks quit modest but it is of the same magnitude as that seen in another physiological cause f cardiac hypert ophy cold-acclimation (see Chapter 4). I is also of the same magnitude as the xercise-induced cardiac hypertrophy eported by those authors who specify that the hearts are trimmed and blotted before weighing (Tipton and Tcheng, 1971 +8% Leon et al 1975: +8%) the importance of these procedures are obvious in view of the increased vascularity and thereby blood content, seen in xercise-induced cardiac hypertrophy (see above).

There appears t be universal agreement that in the rat the cardiac hypertrophy seen after relatively short lasting xposure to various pathological conditions is reversible Thus cardiac hypertrophy due to renal hypertension (Hall et al 1953) hyperthyroidism aortic coarctation and nutr tional anaemia (Beznak et al 1969) isoprenaline treatment (Stanton et al 1969), or simulated altitude (Sizemore et al 1973), egresses on removal f the provoking stimulus. This regression is rapid process with half l fe f appr ximately 7 days (Stanton et al 1969 Sizemore et al 1973) I view f this it is somewhat surprising that cardiac hypertrophy +5% in terms f absolute heart weight, still persists as long as 6 months after cessation f training (paper III) Other investigators (Secher 1923 Hort 1951 Leon and Bloor 1968 Ljungqvist and Unge 1972 and Unge et al 1973) have reported apid return f heart

weight to control values when chronic exercise is discontinued. I paper III young male rats were exercised, while Hort (1951) used old male rats and Ljungqvist and Unge (1972) and Unge *et al* (1973) used relatively adult female rats. Male rats continue to grow throughout their life-span (in contrast to female rats) and in my rats the training period coincides with puberty and the phase of rapid growth of the male rat. The importance of exercise during adolescence and puberty for the development of cardiovascular dimensions i man is well known (see Astrand and Rodahl 1970) and it has been shown that young male rats need less exercise to develop cardiac hypertrophy than adult males, and that they develop a greater degree of cardiac hypertrophy with about the same training intensity (Bloor and Leon 1970). Comparing paper III with the study of Leon and Bloor (1968) who also used young male rats, the training program in paper III is found to be more severe with longer period of training and with a final daily swimming time twice as long perhaps this could account for the difference between my results and those of Leon and Bloor. The study f Sechter quoted above suffers from lack of age-matched controls which makes his conclusions, based on heart ratio unreliable since the heart ratio in the young rapidly growing male rat decreases with increasing age (see control values in Grimm *et al* 1963 Buuck and Tharp 1971 Tharp and Buuck, 1974).

Why then should cardiac hypertrophy induced by exercise in young, still growing, animals persist whereas that induced by the various pathological conditions mentioned above regresses? I adult animals cardiac muscle cell division does not normally occur (Erokhina 1968 Sasaki *et al* 1968 Shafiq *et al* 1968) and the number of cardiac muscle cell nuclei in the adult rat is not altered by chronic exercise (Hort, 1951). Thus in the adult animal hypertrophy of cardiac muscle is produced by ncreased fibre diameter and, when the internal dimensions f the heart are increased, presumably also by increase in length f the individual muscle cell just as normally occurs during postnatal growth this is accomplished without an increase in length of the individual sarcomere (G mm *et al* 1963). Mitosis of cardiac muscle cells does however occur in young rats, the rate of mitosis being quite high at birth and subsequently decreasing with increased age I Sprague—Dawley rats the rate of cardiac muscle cell mitosis is still sign ficantly elevated at the age of 2 months and reaches adult "values" sometime before the age of 4 months (Kl nge and Stocker 1968). Furthermore Rumyantsev and Mirakjan (1968) found that in young male albino rats experimental infarction f the left ventricle, leading to mitral regurgitation could lead to 100-fold increase in mitotic index in cardiac muscle cells in the left au icle. Such post-natal mitoses does not, howeve usually lead t cytoplasmic division (Klinge and Stocker 1968). The possible occurrence of postnatal mitoses in the young rat has to be considered as one explanation for the discrepancy between the workers that claim that exercise-induced cardiac hypertrophy regresses completely (refs. see above) and the f nding f persistent cardiac hypertrophy as long as 6 months after cessation f training reported in paper III. If a detailed comparison is made with the studies of Ljungqvist and Unge (1972) and Unge *et al* (1973) who used the same strai of rats (Sprague—Dawley) as was used in paper III it is found that whereas the rats in paper III were about 6—7 weeks of age at the start of the xperiment, and thus young enough to still have significant occurrence f mitosis in cardiac muscle cells (Kl nge and Stocker 1968) the female rats used by Ljungqvist and Unge (1972) and Unge *et al* (1973) were about 10—11 weeks old at the onset of training, and seem to have been past the age where mitosis in cardiac muscle cells can occur since no mitoses were observed in their rats, neither in control rats, nor in groups with cardiac hypertrophy f various origins (Mendeche *et al* 1973). It is tempting, therefore to speculate that the failure of the xercise-induced cardiac hypertrophy to regress after cessation of training in paper III is due to the presence of an increased number of cardiac muscle cell nuclei and the connection it is noteworthy that the absolute increase in heart weight over the 6 months following cessation of training is virtually

the same in both groups, +0.33 g in control rats and +0.32 g in ex-trained rats. I view of these considerations and in view of the findings of Bloor and Leon (1970) already mentioned above, that very young male rats more readily develop cardiac hypertrophy than adult rats, it would seem possible that in the very young animal hyperplasia of cardiac muscle fibres or possibly occurrence of binucleate cells, as well as hypertrophy may contribute to the adaptive growth of the heart.

Finally I would like to make some comments about the mode of expression of results when studying cardiac hypertrophy It has been claimed that when animals lose weight rapidly following starvation or undernutrition the heart weight decreases roughly in proportion to the decrease in body weight (Jackson 1925 Van Liere and Sleeth 1936 Beznak, 1954) but this has been shown not always to be the case (Oscai and Holloszy 1970) Since the expression of heart weight as fraction f body weight, for example g heart weight/100 g BW makes it easier to obtain statistical significance as the inter individual variation is considerably decreased the use of heart ratio has become popular in the study f cardiac hypertrophy f various origins. This procedure is however only justified when the age and the mean body weight of the groups compared are the same. This is so for the following reasons. Firstly the heart ratio is not constant from birth but rather starts at high value and then gradually decreases (see control values Grimm *et al* 1963 Buuck and Tharp 1971 Tharp and Buuck, 1973) in at least some rat strains plot f ventricular weight versus body weight will fall on straight line from the age when the male rat has reached body weight of about 200 g or more but, due to the slope of the line the heart ratio still decreases with increasing age (see Grimm *et al* 1963) Thus it is not justified to draw conclusions about, for example regression of cardiac hypertrophy on the basis of heart ratio values alone as done by Secher (1923) and Ljungqvist and Unge (1972) in the absence of age-matched controls for comparison. Secondly treatments leading to changes in body weight without actually changing body length do not always lead t parallel changes in heart weight as shown by Oscai and Holloszy (1970) who by inducing weight loss caused marked increases in heart ratio in animals where the absolute heart weight either was unchanged or actually decreased. This point is also illustrated by the increased heart ratio but unaltered heart weight and body length found in rats chemically sympathectomized by guanethidine treatment (see paper VI) Clearly claims of cardiac hypertrophy should only be based on increased size of the heart itself and not on results on heart ratio inflated by loss of body fat!

Adrenal glands

As reviewed in Chapter 1 the increase in weight of the adrenal glands in chronically exercised animals can be accounted for by the increased secretion of ACTH during exercise sessions which induces corticosteroid section and leads to an increase in thickness of the fasciculata zone of the adrenal cortex. In view of the ncreases in the adrenal content of both adrenaline and NA seen in chronically xercised rats (see Section C) the possibility that there is an increase in number and/or size f individual chromaffin cells the adrenal medulla must also be considered since mitoses of chromaffin cells have been demonstrated in he adrenal glands of adult rats (Malvaldi *et al* 1968) although they are normally very infrequent.

The finding that guinea pigs xercised by forced running for five months did not have hypertrophied adrenal glands (Ostman and Sjostrand, 1971) is somewhat surprising in view of the evidence of increases in corticosteroid secretion during exercise in various species (see Chapter 1). It is still conceivable that the lack of adrenal hypertrophy could be due to species difference since the guinea pigs are better adapted to running and will keep running at considerably higher speeds than rats do

(unpublished observation) It is perhaps more likely that they have adapted so fully to the exercise situation that the "stress" of one session per day does not produce a sufficient increase in ACTH secretion to give rise to an increase in the weight of the adrenal cortex. It is noteworthy that these guinea pigs had been subjected to regular exercise for a period of five months, much longer than the period of training in most studies. In this context some longitudinal studies are of interest and give some support to the above hypothesis. The difference in plasma corticosterone levels between exercised rats and controls reaches a maximum some time between 2 and 4 weeks after initiation of training and subsequently decreases again so that resting plasma levels of corticosterone are normal 6 to 8 weeks after the onset of regular exercise although at that stage adrenal cortical hypertrophy persists (Frenkl and Csalay 1962, Tharp and Buuck 1974). When trained and untrained animals are submitted to the same work-load the blood level of steroids increases more in the untrained than in the trained rat and the same is true for humans (Frenkl *et al.* 1968, 1969). Furthermore, plasma levels of corticosterone stay elevated after exercise for much longer in untrained (more than 3 h) than in trained (45 min) rats (Frenkl *et al.* 1969). All these studies suggest that the exercise-induced secretion of ACTH, and thereby the stimulus for adaptive growth of the adrenal cortex, is highest early on during the training period, and subsequently decreases considerably and perhaps in the "ideally trained" state disappears completely. A further observation that suggests that the guinea pigs were not subject to much "stress" is the fact that they did not have a significantly increased catecholamine content in the adrenal gland (Ostman and Sjostrand, 1971).

As regards the argument of which type of exercise, treadmill running or swim training, is the less stressful form of exercise, the finding that the adrenal hypertrophy is so much greater in the rats exercised on the treadmill than in the swim-trained rats gives further support to the suggestion (see Chapter 2) that in rats swim training is the form of chronic exercise that involves least stress.

Spleen (papers III and V)

Alterations in the weight of the spleen following chronic physical exercise appear to have been previously reported only by Hatai (1915) and Petow and Siebert (1925). The former found a decrease in size of the spleen in exercised rats, and the latter authors reported a 19% lower spleen weight in rats that ran for 320 min/day at a speed of 6.5 m/min, whereas there was no change in splenic size in rats running 20 min/day at 36 m/min. Petow and Siebert (1925) did not comment on this finding however. There appear to be two possible explanations for a change in weight of the spleen. Firstly, since the spleen is a blood-rich organ it is possible that unequal degree of vasoconstriction in the different groups of rats could lead to an apparent change in organ size. Secondly, the amount of actual tissue stroma, most notably lymphoid tissue, can be decreased. Considering the first alternative, it is theoretically possible that this could account for a difference between controls and trained rats since vascular smooth muscle in trained rats exhibits an increased sensitivity to the vasoconstrictor actions of NA (paper III, Section G). On the other hand the impulse activity of sympathetic nerves in the spleen, as estimated by the turnover of NA, is significantly lower in trained rats than in the controls. There appears to be no reason to expect that the method of killing used in paper V, decapitation, should cause a more marked increase in nervous activity of sympathetic nerves to the spleen in trained rats than in controls and thus the first explanation of the decrease in spleen weight is unlikely to be correct. Furthermore, the exercise-induced decrease in spleen weight is seen also in chemically sympathectomized rats (paper VI). The second alternative, a decrease in lymphoid tissue, seems to have more arguments to support it.

Thus regular administration of steroids has been found to lead to decrease in splenic weight (Mokumut *et al* 1980 Wurtman 1966) due t decrease in lymphoid tissue. This mechanism could then explain the decrease in weight of the spleen found in chronically exercised rats since there is much evidence that physical exertion leads to increased secretion of corticosterone in rats (see above) Such a mechanism could lso account for the otherwise puzzling results of Petow and Siebert (1925) since the 20 min period xercise might have been too short to produce high levels of corticosterone in the plasma.

Salivary glands will be discussed in Chapter 4

SECTION C: CATECHOLAMINE CONTENT IN VARIOUS ORGANS

(Papers I II III, V unpublished observations)

RESULTS

Brain

In the brains of chronically xercised rats, killed 48 h after the last period of xercise both the NA content and the NA concentration were consistently and significantly increased. In one group, killed after 17 weeks of daily swim-training the NA content of the whole brain was 26% higher than that of sedentary controls ($p < 0.001$ paper V). I another group which was studied after 14 weeks of daily swimming, the NA content in the brains of trained rats was increased by 14% (0.73 ± 0.02 μg NA, 12, as compared to 0.64 ± 0.02 in controls, = 11 $p < 0.01$) The DA content f the brain, on the other hand, was not significantly altered (paper V).

Heart

Total cardiac NA content has been found to be increased fter chronic xercise (papers I 14%, n.s., II 25%, $p < 0.001$ III +26%, $p < 0.01$ V: +22%, n.s.) but the increase was only significant in swim-trained rats and not in the study using treadmill running (paper I) The concentration of NA in the heart was likewise increased, although to somewhat smaller extent due to the concomitant cardiac hypertrophy (papers I +6%, s., II 16%, $p < 0.01$ III 18%, $p < 0.05$ V: +7%, s.).

Salivary glands

NA content and concentration in submandibular glands were not significantly altered (paper III).

Spleen

The total NA content of the spleen was increased by 26% after 14 weeks of swim-training (paper III $p < 0.01$) no significant increase was however found in the study after 17 weeks of swim-training (paper V).

Adrenal glands

The adrenal NA content was increased I the trained animal in all the studies, but the increase was more pronounced in the studies tilizing swim-training (papers II +94%, $p < 0.001$ III +32%, $p < 0.01$) than in the study using treadmill running (paper I: +24%, .s.). The adrenal adrenaline content was increased by a similar degree in all the studies on trained rats, whether trained by running or by swimming (papers I +21%, $p < 0.01$ II +20%, $p < 0.05$ III +13%, $p < 0.01$) In swim-trained rats, but not in rats trained by running on a treadmill the increase in NA content was relatively greater than the increase in adrenal ne content, so that there was an increase in the proportion of catecholamines comprised by NA (paper II 22 ± 1% compared to 15 ± 1% in the controls $p < 0.001$ paper III: 16.1 ± 0.7% as compared to 13.9 ± 0.7%, $p < 0.05$).

Thus, in chronically xercised rats the stores of transmitter are increased I noradrenergic neurons I brain heart and possibly spleen and in the chromaffin cells in the adrenal medulla

DISCUSSION

The effects of chronic exercise on the amounts of CA in various tissues can best be interpreted in terms of the general hypothesis that chronic increase the activity of a neuron leads to changes in its biochemical composition some f which may be truly adaptive in the sense that they enable the neuron better to fulfill its physiological function One of the earliest demonstrations f this principle were the morphological studies of motor and sensory neurons subjected to a sustained increase in impulse flow which showed that there was an initial depletion of Nissl substance (RNA + protein) followed by morphological and biochemical evidence of increased nuclear and nucleolar activity (Hydén 1943) After prolonged increase in impulse flow in chronically stimulated vestibular neurons, Hydén and co-workers demonstrated an increase I RNA and protein content and I cytochrome- xidase and succinoxidase activity (see review by Hydén 1967). Meerso and Kroichina (1966) found that neurons of the intramural ganglia in the heart ndergoing hypertrophy following experimental aortic stenosis as reflected by their morphological appearance appeared to undergo similar sequence of events terminating in stage of "stable hyperfunction with an hypertrophied neuron with increased total content f Nissl substance. In cholinergic neurons it has been suggested that an increase in the level of choline acetyltransferase activity reflects increased neuronal activity (Oesch, 1974 see lso Ekstrom 1975) However the biochemical events that follow increase in fferent nerve mpulse flow have been studied in greater detail in sympathetic neurons and In particular in the adrenal gland

Adrenal gland

Enzymes of catecholamine biosynthesis. In the adrenal gland the activities of three f the enzymes involved n CA biosynthesis have been shown to be increased after various stressful stimul The enzymes are tyrosine hydroxylase the presumed rate-limiting step (Bertler t al 1960 Levitt t al 1965) dopamine β–hydroxylase   characteristic component of adrenergic storage vesicles (Smith, 1972), and phenylethanolamine N–methyltransferase (PNMT) the enzyme converting NA to adrenaline Rises the activities of all three enzymes occur in cold stress (Thoenen, 1970 Kvetnansky *et al* 1971[a]), immobilization stress (Kvetnansky *et al* 1970 1971[b]) as well as in "psychosocial stimulation" (Henry *et al* 1971) Similarly increases in tyrosine hydroxylase and dopamine β–hydroxylase activity in the adrenal gland have been shown 24–96 h after insulin-induced hypoglycaemia (Viveros *et al* 1969

Patrick and Kirshner 1971). PNMT was not studied in these experiments. All these are conditions that increase splanchnic nerve impulse flow (see below). The increase in tyrosine hydroxylase activity can be prevented by denervation of the adrenal gland, but can be induced in denervated adrenal by means of repeated acetylcholine administration (Patrick and Kirshner 1971). Furthermore the long-term rise in tyrosine hydroxylase activity occurring after prolonged (at least 4 h) increase in splanchnic nervous activity is evident after 24 h but reaches maximum after 48 h and is abolished by blocking transcription and/or protein synthesis (see reviews by Thoenen *et al* 1973 and Kirshner 1975). Consequently this increase in tyrosine hydroxylase activity is presumed to be induced by increased presynaptic release of acetylcholine and be due to *de novo* synthesis of enzyme. Increase in dopamine β-hydroxylase activity in adrenal glands also appears to be predominantly regulated by increase of splanchnic nervous activity and be mediated through *de novo* protein synthesis, although ACTH may serve a permissive role (see review by Axelrod, 1972). PNMT however seems to be controlled by steroids from the adrenal cortex (Wurtman and Axelrod, 1966; Wurtman 1966) as well as by neural factors (Re *et al* 1971; Kvetnansky 1973).

Conditions leading to increase in adrenal catecholamine content. Increased adrenal adrenaline content has been observed in various chronic physiological and emotional stress situations such as cold acclimation (Refs. see Chapter 4), grouping (Welch and Welch, 1968; Welch and Welch 1969), daily fighting (Welch and Welch 1969) and "psychosocial stimulation" (Henry *et al* 1971); all these are conditions which on acute exposure are known to increase urinary adrenaline excretion and/or cause acute decrease in adrenal adrenaline content (cold: see Chapter 4; grouping: Welch and Welch, 1968; fighting: Welch and Welch 1969).

Similarly increased adrenal NA content has been reported in some but not all studies on cold acclimation (for references see Chapter 4) and after "psychosocial stimulation" (Henry *et al* 1971).

Thus there is abundant evidence that increased afferent nervous activity in the adrenal medulla causes adaptive responses in the chromaffin cell leading to increased synthesis capacity (as indicated by increased activity of tyrosine hydroxylase and PNMT) as well as possibly an increased storage capacity (as indicated by increased dopamine β-hydroxylase activity) for the catecholamines.

It can be concluded that the observed increases in adrenal CA content in chronically exercised animals is an adaptation to the increased secretory activity of the adrenal medulla. That trained rats have an increased storage capacity for CA in their adrenal glands is also corroborated by Roffman *et al* (1973) who found that repeated swimming sessions lead to an increase in adrenal dopamine β-hydroxylase activity.

Exercise and adrenal catecholamine turnover. That adrenal adrenaline synthesis and turnover are increased during prolonged exercise in a rotating drum has been shown previously (Gordon *et al* 1966[b]) but there seems to have been no studies on adrenal NA turnover during exercise. It is well known that urinary NA, as well as adrenaline excretion (refs. see Section D) and plasma NA levels (see review by von Euler 1974) are increased during exercise but in view of the concomitant increase in sympathetic nervous activity the adrenal contribution can only be assessed by direct turnover studies. However the increase in adrenal NA content found in the swim-trained animals (papers II, III) suggest that adrenal NA turnover is also significantly increased at least during strenuous physical exercise perhaps as a secondary defence when the transmitter stores in peripheral sympathetic nerves threaten to get exhausted as occurs in the heart in untrained rats after prolonged swimming (paper II).

Treadmill running compared with swimming. There is somewhat different pattern of increase in adrenal CA content in the rats trained on a treadm ll (paper I) compared to rats trained by swimming (papers II III) since in the rats trained by swimming there was a significant increase in the proportion f the catecholamines comprised by NA. This difference in the response of the adrenal medulla to the different types of training could be due to the fact that running on treadmill with an electrical fence certainly would appear to constitute much more of an emotional stress than swimming does while the swim-training used on the other hand probably constitutes a greater working load. It is well known that "emotional" stress is the more potent stimulus for adrenal adrenaline secretion (Kvetnansky and Mikulaj 1970 see review by Frankenhaeuser 1971) The absence of significant increase in adrenal adrenaline content seen in guinea pigs trained by treadmill running (Ostman and Sjostrand, 1971) could be due to species differences or to the fact that they were less emotionally stressed by the runn ng than the rats were since an lectrical fence was not used and ince they are physically much better adapted to running than rats are A third possibility since the guinea pigs were trained for longer period of time than any rat group (22 weeks as compared to 14 to 15 weeks) and attained higher increase cardiac NA conce tration (21% as compared to 6%, 16% and 18% in rats) is that as th peripheral sympathetic nervous system gets progressively more adapted to the demands f exercise there is progressively less need for supplementary adrenal secretory activity t is noteworthy that in the guinea pigs there was also an absence f adrenal hypertrophy (Ostman and Sjostrand, 1971).

Brain

Many xperimental stimuli, that during acute xposure increase the turnover of NA in whole brain or part of the brai (for references see section F and paper V), when intense cause an acute decrease in the NA content of the brain this has been shown with severe cold stress (Lew and Maynert, 1962 Maynert and Lew 1964 Stone 1970) with forced running (Gordon *et al* 1966[b] Stone 1971) grouping f previously isolated mice (Welch and Welch 1968 Welch 1967[a]) aggregation f strangers (Bliss and Ailion 1969) and in fighting mice (Bliss and Zwanziger 1966 Welch and Welch 1969) However when the stimulus is chronic, or intermittent but repeated regularly over prolonged period of time an elevation of NA content the brai occurs, as seen in prolonged cold posure (Ingenito and Bonnycastle 1967) after two weeks exposure to da ly fighting (Welch and Welch, 1969) after prolonged "grouping (Welch 1967[b]) and after repeated electroconvulsive shocks (Kety *et al* 1967). Thus the finding of an elevated NA content and concentration in the brain f chronically xercised rats fits well with the changes reported in both physiological and unphysiological' types f stress. It should be emphasized that whole brain NA content is a very crude measure and that the elevation of NA content in the brains f chronically exercised rats may be l mited to one few areas f the brain perhaps th hypothalamus and/or the brain stem the areas that show increased NA turnover during xercise (Stone 1971 Gordon *et al* 1966[b])

The nderlying mechanism of this increase is likely to be the induction presumably secondary to increased impulse flow in the neuron of the enzymes of the biosynthetic pathway for the transmitter as also occurs in peripheral noradrenergic neurons and adrenal medulla (see above for references) Thus prolonged cold exposure leads to an increase in tyrosine hydroxylase activity which initially is seen only in the medulla oblongata (Thoenen 1970) where the cell bodies occur of many of the nor adrenergic neurons that send terminals to the hypothalamus (Dahlstrom and F xe 1965 see review by Bolme *et al* 1972) which is one area where there is pronounced increase in NA turnover during cold stress (Simmonds 1969) After time lag, presumed to be due to axonal transport f the enzyme

there is an increase in tyrosine hydroxylase activity in other brain areas as well (see Costa and Meek, 1974). Tyrosine hydroxylase activity in the brain has also been shown to be increased by other conditions leading to increased NA content in the brain such as electroconvulsive shock (Musacchio *et al* 1969) and immobilization stress (Lamprecht *et al* 1972). However the best indication of increased storage capacity is an increase of dopamine β-hydroxylase activity. It would be of interest to study dopamine β-hydroxylase activity in the brain in physiological conditions which elevate brain NA content, but this seems not to have been done as yet. However chlorpromazine treatment which is known to lead to increased NA turnover in the brain (Corrodi *et al* 1967, Nyback and Sedvall 1970) has been shown to increase dopamine β-hydroxylase activity in the rat brain upon chronic administration (Wise *et al* 1974).

The finding that the DA content in whole brain is not increased in chronically exercised rats is not surprising since the synthesis (Gordon *et al* 1966[b]) and turnover (Ostman and Nyback, previously unpublished, see Section F) of DA in whole brain has not been found to be increased by physical exercise.

Heart

Induction of enzymes and increased storage capacity. The increases found in both the content and concentration of NA in the heart are likely to be the result of an adaptive response to daily periods of markedly increased nervous activity just like the adaptive response in the brain and in the adrenal medulla. Marked increase in cardiac sympathetic activity as estimated by NA turnover studies, has been shown to occur both during running (Gordon *et al* 1966[b], Sheldon *et al* 1975) and during swimming (paper II) and nerve impulse-induced increases in tyrosine hydroxylase levels after combined cold and swimming stress takes place in rat stellate ganglia and heart just as in the adrenal medulla (Thoenen *et al* 1973). Furthermore Roffman *et al* (1973) have shown that chronic, but not acute swimming stress leads to increased dopamine β-hydroxylase activity in cervical sympathetic ganglia. Increased tyrosine hydroxylase activity in the heart would suggest that there is increased maximal synthesis capacity in the "athletic heart". Paper II provides some support for this assumption as the trained rats were able to maintain their (higher) stores of NA throughout 8 h of continuous swimming, with an absolute turnover of NA of 0.164 μg NA/heart/h during the 4–8 h period whereas the untrained rats were unable to maintain their cardiac stores of NA during the same interval. As the turnover was higher in the untrained rats they would have needed 0.236 μg NA/heart/h to maintain their stores but after 4 h the maximal synthesis rate evidently was only 0.146 μg NA/heart/h and consequently there was a drop in cardiac NA content. It can be seen therefore that 0.164 μg NA/heart/h was a submaximal synthesis rate for trained rats, whereas in untrained rats a synthesis rate of 0.146 μg NA/heart/h was an absolute maximum.

As mentioned above dopamine β-hydroxylase is almost exclusively confined to noradrenergic storage vesicles, and a rise in the level of dopamine β-hydroxylase would thus seem to indicate the presence of an increased number of NA storage vesicles or perhaps less likely an increase in dopamine β-hydroxylase content per storage vesicle.

Does neoformation of nerve terminals occur? Whether the storage vesicles are contained within the same number of terminals or whether the number of noradrenergic terminals is increased as well is not yet absolutely clear. Unge *et al* (1973) reported a normal number of noradrenergic axons but an increased number of noradrenergic varicose fibers in the hearts of chronically exercised rats. They

suggested that this increase was related to the well-known increase i capillary density i the heart of chronically exercised animals (see Section B for references) because each cardiac capillary is normally accompanied by at least one noradrenergic nerve fibre (Unge *et al* 1973) This proposal fits with what is known about the capacity of the noradrenergic neuron to extend its field of inner vation (see Burnstock and Costa 1975) and with the finding that, at the site f capillary neoformation in the hearts of xercised rats there are varicose nerve fibres in close proximity to the dividing pericyte (Unge *et al*., 1973) (In this connection it is of interest that Sm th and Wolpert, 1975, have suggested that the presence of nerves may actually be necessary for the formation of new blood vessels.)

Since especially in the atrium, the number of noradrenergic varicose nerve fibres considerably exceeds the number of capillaries (Dahlstrom *et al* 1965) one would not xpect the same proportional increase in NA content as in capillary density Thus, with swimming sched le less severe than ours Leon and Bloor (1968) and Bloor and Leon (1970) f und increases in capillary/fibre ratio of 40—44% in swim-trained rats.

Furthe support for the concept of neoformation of adrenergic nerve terminals adaptive cardiac hypertrophy is the report by Borchard (1978) that in hypertrophied human myocardium the density f nerve terminals is higher than would be expected f unaltered umber of terminals was simply d luted" by hypertrophied muscle cells.

It can be concluded that the rise in the content and concentratio f NA in the hearts f chronically xercised rats may well be accounted for by an ncrease in the number of noradrenergic nerve terminals each with perhaps relatively normal content f storage vesicles.

Factors determining the degree of increase in cardiac noradrenaline content. It is noteworthy that there does not seem to be very close correlatio between the degree f cardiac hypertrophy and the degree f increased cardiac NA content in all our training exper ments where rats were studied (papers I, II III V Section E) the cardiac hypertrophy obtained was consistently from 7 t +8%, whereas the ncrease in t tal NA content in the heart varied twofold between 14% and 28%. It seems that the increase in cardiac NA levels obtained with regular physical xercise is related to several factors described below

First, the *intensity* f the precipitating st mulus. Thus, the cardiac hypertrophy obtained in the cold-acclimated rats was the same 7%, as in the xercised group but the increase t tal NA content the heart was nly +10% compared with +25% in the exercised group (paper III see Chapter 4).

Second the *daily work load* Thus Leon *et al* (1975) who like us used young male rats but swam them for 1 h/day nly 5 days/week for 12 weeks found no significant increase in total cardiac NA content (+5%) but virtually identical degree of cardiac hypertrophy (+8%)

Third, the *frequency* of the st mulus. Thus, intermittent swim-training, 3 times/week but with the same daily swimming time leads to smaller increase in total cardiac NA content in spit of the same degree f cardiac hypertrophy (+8.5%) (see Section E in this chapter)

Fourth possibly also the age f the an mals. The failure to obtain ignificant increase in cardiac NA content in the run-trained group could be related to the fact that not only is the xercise probably less intense than the swim-training but also t the fact that these rats were somewhat (3 weeks) older as well In this respect it is f relevance that Bloor and Leon (1970) found that the same type f swim-training caused comparatively larger increase in ventricular dry weight and in capillary/fibre ratio in one-month old than in three-month old rats (see also Sectio B)

Results of other workers. Our findings on the cardiac NA content and concentration in chronically exercised rats would seem to be at variance with the work of De Schryver and co-workers (De Schryver *et al* 1967 1969 De Schryver and Mertens–Strythagen 1972) who claim that the bradycardia of the athletic heart might be related to their finding of decreased NA concentration in the hearts of "exercised" rats. Their exercise program is however of very low intensity running speed of 8.3–12.5 m/min (about one third to one fourth of the running speed used by other workers studying the athletic heart (Becher 1921 1923 Hort 1951 Van Liere and Northup 1957 paper I) and they exercise their rats only three times per week. Such training parameters are not sufficient to induce cardiac enlargement (Petow and Siebert, 1925 Leon and Bloor 1968 Bloor and Leon 1970 Hecht *et al* 1973) and thus it is hardly surprising that De Schryver and his associates never find any cardiac hypertrophy in their "trained" rats. Since the athletic heart by definition is an enlarged heart, De Schryver and co workers have obviously not been studying this condition but a different experimental situation. Furthermore since the occurrence of significant bradycardia in trained rats seems to be associated with the occurrence of cardiac hypertrophy (see Chapter 1) and since De Schryver *et al* never actually measured the heart rate in their rats their conclusion – "this decrease of sympathetic transmitter substance might explain in part the bradycardia found in the athletic heart (De Schryver *et al* 1967 1972) must be rejected.

However their report itself that during their experimental conditions with such mild and intermittent exercise program they see a decrease both in total content and in concentration of NA, lasting up to 6 days after cessation of exercise seems very puzzling, particularly since they report that, in contrast three days after a single bout of exercise in untrained rats their cardiac NA concentration was in fact 7% (non-significantly) higher (De Schryver *et al* 1969) The reports of De Schryver *et al* are also at variance with that of Hecht *et al* (1973) who reported that with a similar low intensity exercise (running speed 6 m/min) but a daily period of running increasing up to 7 h/day there was as in the studies of De Schryver *et al* an absence of cardiac hypertrophy but there was no significant change in cardiac NA concentration indeed it tended to be higher (non-significantly) in the trained rats. It seems somewhat unlikely that strain differences could explain the discrepancy in results between De Schryver and co-workers (who used Wistar rats) and other workers in the field, since in eight other publications using three different strains of albino rats (unspecified albino rats, Charles River C.D. rats Sprague–Dawley rats) as well as guinea pigs and various training schedules varying from mild to heavy there is no other observation even of a non-significant decrease in total NA content of the heart of chronically exercised animal (papers I, II III V Section E this chapter Ostman and Sjostrand, 1971 Hecht *et al* 1973 Leon *et al* 1975)

Furthermore there are several obscure aspects of the reports from De Schryver *et al*. Firstly they state in all their publications that "the growth curves were the same in controls and trained animals as judged from their published data however this growth curve must be highly abnormal since the reported weight increase in their male rats during an experiment lasting 3 months is about 90 g, with a range of 40–110 g (De Schryver *et al* 1969) A similar very low increase in body weight, averaging 80 g, during the three month experimental period can be deduced from their previous data (De Schryver *et al* 1967) if one uses the subsequently published heart ratios to calculate the final body weight which is not given in that publication. Young male rats normally grow very rapidly thus for example rats of the Kyoto–Wistar strain and of ordinary Wistar strain with the same initial body weight as the ones used by De Schryver and associates would be expected to gain about 165 g and 250 g respectively over a 12 week period (see Ozaki *et al* 1972 and Tikutsu and Kashu 1972) Similarly young rats of our strain of Sprague–Dawley rats would gain about 270 g over 3 months (paper VI) as would Charles

River C.D. rats (Leon *et al.* 1976). Both these strains and the Wista rats of Ozaki *et al.* (1972) reach body weights of the order of 400—490 g at the same age as that when the rats of De Schryver *et al.* are reported to weigh about 240 g. Thus, either the growth of the rats employed in their studies is severely stunted by some aspect of their experimental conditions or their experiments were not designed in proper longitudinal fashion. If one uses the data on heart weight and heart ratio given by De Schryver and Mertens—Strythagen (1972) to calculate the mean body weight of the different experimental groups it is found that, for example, the mean body weight of the 600 m/h group killed after one week of training is 258 g whereas the mean body weight of that killed after 8 weeks of training at 600 m/h is 232 g! Male rats continue to grow throughout life and it is inconceivable that young male rat maintained with a reasonably adequate food supply should fail to gain weight, and instead lose weight, over a period of 7 weeks. Thus, one is forced to conclude that a false impression is given in their Methods description of a "longitudinal" design of the experiment (e.g. "at the beginning of the experiment the rats were divided into several groups" De Schryver *et al.* 1972) and that in fact some other experimental design has been used, resulting in all experimental groups being of roughly the same body weight at death regardless of the duration of training. This is borne out by the fact that in a study involving 20 different experimental groups there is only *one* single group of untrained animals used as controls for experiments spanning from periods of one to nine weeks training in the motor-driven drum. Such an experimental design would mean that none of the exercised groups were comparable to each other since they would all have started the exercise at a different age. All these aspects of the work of De Schryver and his associates would suggest that they have not used adequate control groups to exclude that their reports of decreased NA content and concentration in the hearts of their "exercised" rats is not in fact an artefact derived from their experimental design.

In summary therefore it would seem that, (disregarding the work of De Schryver and associates) there is no discrepancy between the findings of different workers on the NA content and concentration in the heart of chronically exercised animals that cannot be accounted for by the use of different training parameters.

Long term effects of exercise. What happens to the sympathetic innervation of the heart in a chronically exercised rat that is then allowed to rest for a prolonged period? In paper III it was found that although there was still a significant cardiac hypertrophy (expressed as heart ratio) the cardiac total NA content and NA concentration were no longer significantly different from untrained controls six months after the last exercise period. The lack of statistical significance was due to a large inter-individual variation in the two groups, both the NA content (+26%) and the NA concentration (+18%) of the heart were, in fact, considerably higher in the ex-trained group than in the controls. Inter-individual variation in body and organ size increases with age in the rat and if the very highest and the very lowest observations in both of the two groups of rats studied 6 months after cessation of exercise are excluded it is then found that total NA content (controls: 1.16 ± 0.12 μg NA, ex-trained: 1.60 ± 0.07 μg NA; $p < 0.05$) is probably significantly higher in the ex-trained rat. As regards the NA concentration in the heart (controls: 0.74 ± 0.08 μg NA/g, ex-trained: 0.93 ± 0.04 μg NA/g; $0.05 < p < 0.1$) the remaining difference only just falls short of statistical significance. This is in some contrast to the report of Unge *et al.* (1973) that in their female rats there did not seem to be an increased density of noradrenergic terminals in the heart two months after cessation of training. However, probably due to different experimental conditions, as discussed earlier (Section B), they do not see a persisting cardiac hypertrophy either, which is seen in our male rats. Assuming that at least part of the increased cardiac NA stores in trained rats is due to formation of new terminal nerve fibres accompanying

vascular neoformation as discussed above, it seems rather unlikely that sympathetic varicose fibres having once developed should degenerate on cessation of physical exercise in particular since the increased capillary density induced by physical exercise persists for at least two months after the regular exercise is terminated (Ljungqvist and Unge 1972). A continued presence of an increased number of varicose nerve fibres would not of course mean continued presence of an increased total NA content unless the NA content f each varicose f bre was maintained. I the absence f continued periods of swimming there would be no further stimulus, through increases in nerve impulse flow to induction f synthesis f highe than normal amounts of tyrosine hydroxylase and dopamine β-hydroxylase. Thus one might speculate that there may be an intrinsic mechanism operating in the neuron which prevents the transmitter concentration in each varicose nerve fibre as distinct from each neuron, from falling below certain minimum, and that this mi mum is not greatly different from that seen in normal control rats.

Submandibular gland

The total content and concentration f NA in the submandibular salivary glands were not sign f cantly influenced by chronic physical exercises as might be expected since this organ is not directly involved in the cardiovascular adjustments to physical work.

Spleen

The results on total content f NA in the spleen in papers III and V are somewhat conflicting in that whereas in paper III there a significant increase in total NA content (26%) there was only non-significant increase f 3% in paper V The reasons for th discrepancy are not clear it seems very unlikely that the more variable recovery of the assay procedure in paper V could on its own account for such a diminution of differential Possibly time factor could be involved since the rats in paper III had been swim-trained for 14 weeks, with the daily swimming period increased to 2 h/day and the rats in paper V on the other hand had been trained for 17 weeks with the daily xercise period increased to 2½ h/day Could t be that the development in trained ats f increased sensitivity t the vasoconstrictor actions f NA (see Section G) so diminishes the demand for increased sympathetic arous activity in the spleen during exercise that th stimulus for increased storage f NA in splenic nerves disappears? It is noteworthy that the activity f sympathetic nerves in the spleen f the trained rats in paper V is in fact markedly decreased during resting condi ions, which would tend to lend some support to the above interpreta ion

There is l ttle doub however that the concentration f NA in he spleen emains elevated, since if the data on NA concentration in the spleen f om paper V are pooled with data from other rats out of the same experimental groups used for her experiments one finds value of 0.94 ± 0.06 μg NA/g for control ats (n 16) and 1.14 ± 0.06 μg NA/g for trained rats (n 15 $p < 0.02$) It can be argued, though that the NA concentration f the spleen in this case is rathe irrelevant since t probably results from decrease in the amoun f lymphoid ssue in the spleen (see Section B)

SECTION D URINARY EXCRETION OF CATECHOLAMINES AND VANILLYL MANDELIC ACID

(Papers II III VI and unpublished data)

RESULTS

At rest

I paper II where the trained rats were rested fo 24 h before the collectio of urine, no difference was found between trained and untrained rats as regards th excretion of NA nd adrenaline in the urine at rest In paper III however where the collection of urine was preceded by 48 h rest in both groups (the control rats having had their one and only bout f swimm ng for the purpose f urine collection 48 h earlier) th urinary excretion f NA in trained nimals was significantly lower tha that of controls being 67% of the control value ($p < 0.001$) while the xcretion f adrenaline in the urine was not significantly different from that of controls.

After exercise

I untrained rats 2 h period of swimming was found significantly to increase the urinary xcretion of both NA (II +124%, $p < 0.001$ III +44%, $p < 0.001$) and adrenaline (II: +300%, $p < 0.001$ III 38%, $p < 0.05$) during the 22 h following the swimming session. I the trained groups the excretion f NA in the urine was lso significantly elevated after xercise but the rise was smaller both in absolute terms (µg/22 h) and as proportion of the resting value. Thus in trained rats the urinary xcretion of NA fter xercise was only 64% ($p < 0.001$ III) to 65% ($p < 0.01$ II) of that of th respective control groups. The rise in the excretion of adrenaline in the urine fter exercise was lso significantly smaller in tra ned than in untrained rats in both studies in paper II the net ncrease in adrenaline xcreted was only 33% of that seen in control rats, wh le in paper III the rise in adrenaline excretio fter exercise was not even significant in the trained group The proportion of total catecholamines xcreted in the ur ne that was constituted by NA was however not significantly changed after exercise in either trained or untrained rats (paper VI)

Excretion f vanillyl mandelic acid

During resting conditions xcretion f vanillyl mandelic acid (VMA) in th urine was lower in the trained group being 23.5 ± 1.2 µg VMA/22 h (n 12) as compared with 27.5 ± 2.0 in the control rats (n 12) $p < 0.05$ (previously unpublished data)

Several months after cessation f exercise (paper III)

6 months after cessation f xercise there is no significant difference between x-trained rats and their age-matched control group in the excretion of NA and adrenal ne in the urine at rest and during cold stress. After xercise however the -trained rats still xcret less NA (−16%) than untrained rats do ($p < 0.05$) Unlike the situation in fully trained rats the excretion f adrenal ne in the ur ne fter exercise is the same in trained rats as that found in controls. The persistence x-trained rats f lower degree of overall sympathetic act vity during exercise as monitored by he urinary xcretion of NA, is also ver fied when the net increase in the amoun f NA xcreted by individual rats was

determined in paired observations (controls: 0.76 ± 0.10 μg NA/22 h 10, ex-trained: 0.41 ± 0.07 μg NA/22 h 11 $p < 0.02$)

DISCUSSION

Our finding that exercising rats by swimming leads to an increased excretion f NA and A in the urine confirms earlier findings on humans (see Historical Introduction and review by Euler 1974). Since in contrast to the above mentioned studies, we were not ble to collect urine formed *during* the performance of the exercise we studied the excretion of CA in the urine soon fter the peak levels f catecholamines i plasma must have occurred and during the subsequent 22 h period of recovery and rest.

One cannot be completely certain that all the rats had empty bladders after swimming (see Chapter 2) but, assuming that most f the urine formed during swimming was lost in the swimming bath it is likely that the increase in urinary CA excretion evoked by xercise was greater than that actually measured. A second factor which makes it difficult to compare the present results with those obtained in humans is the fact that the rate f CA xcretion was measured over 22 h period while in humans it can be measured over much shorter intervals. Karki (1956) suggested that in man the urinary excretion of catecholamines returns to normal fter physical exertion in about one hour this is probably not quite the case in rats, since ur results both fter exercise and cold exposure suggests that it takes more than 24 h for rat to reach truly basal excretion values f NA after these two types of physiological stress (see later in Discussion and Chapter 4). However in order to make very rough comparison between the species, one can allocate the net increase in catecholamine excretion occurring after exercise in rats t the 2 h immediately following the swimming period, and compare that rate of catecholamine excretion (µg/h) with the normal resting excretion rate for each group xpressed as μg/h th gives t least very approximate idea f the severity of the work load although, due to the loss f urine during swimming, it would tend to underestimate the increase in catecholamine xcretion in the rats. Using the data from paper III, it is then calculated that control rats have bout six-fold increase in urinary excretion of NA, nd about five-fold increase in adrenaline excretion, immediately after exercise as compared with excretion at rest while the corresponding values for trained rats are five-fold increase in NA, and three-fold increase in adrenaline, xcretion in the urine. This magnitude of increase suggests that the work load involved in swimming is considerable, since in humans it needed work load f more than 80% f the individual maximal xygen uptake to increase the arterial plasma concentration of NA more than five-fold (Haggendal *et al* 1970) and since it is known that although supramaximal work in humans can increase plasma NA concentration 80-fold already 5 mi after work the plasma concentration f NA has come down to 10–12 times the resting value (Johnson *et al* 1974).

Excretion of adrenaline

There was no difference between trained and untrained rats in resting xcretion of adrenaline (papers II and III). The absolute values of resting xcretion of adrenaline, however were considerably lower in paper II than in paper III although the amounts xcreted after exercise were about the same in the two studies. The xcretion of CA in the urine is known to vary according to the time of year (Leduc, 1961) and th is probably the xplanatio for he difference in resting CA excretion in the two studies. It is noteworthy that when the resting xcretion of adrenaline is comparatively high as in paper III, there no further statistically significant increase in the urinary xcretion of adrenalin fter exercise in the trained group, whereas when the resting xcretio is comparatively low as in paper II, xercise increased the secretion of adrenaline even in trained rats.

I human subjects, who are motivated and therefore probably not too emotionally stressed by an xercise session physical xertion increases the plasma levels, as well as the urinary xcretion f both NA and adrenaline (for references see Chapter I) emotional stress, on the other hand causes rises mainly

In the urinary excretion of adrenaline both in humans (see review by Frankenhaeuser 1971) and in rats (Kvetnansky and Mikulaj, 1970) Therefore the finding that trained rats exhibit a much smaller rise in urinary excretio of adrenal ne fter exercise then control rats do while the resting adrenaline excretion remains the same as that of controls, could at least partly be due to an adaptation to the emotional stress of swimming. Such an adaptation is however unl kely to be the sole factor involved, since intermittently xercised rats, well adapted to the stress of swimming, still excrete more adrenaline in their urine after xercise than daily exercised rats do, although they excrete significantly less than control rats do (see Section E) This points to the existence of adaptive changes in adrenal ne secretion induced by the physical exertion itself Such changes are not likely to be predominantly secondary to changes in tissue sensitivity to the calorigenic" actions of adrenal ne since the adrenaline excretion both during rest and during cold stress, potent stimulus for adrenaline secretion, is th same in trained rats as in controls (see also Chapter 4)

It also deserves to be pointed out that fter exercise there was a parallel increase in urinary excretion of NA and adrenaline even in the untrained rats so that the proportion of NA was unchanged from rest (87.5 ± 0.8%) compared with after exercise (88.3 ± 1.0%) (paper VI) Thus it seems likely that physical xertion, rather than emotional stress, was the most prominent factor causing the observed activation of the sympatho-adrenal system during swimm ng even in the untrained rats unaccustomed to water

Excretion of noradrenaline

The discrepancy between paper II and III on the question of whether or not trained rats xcrete less NA in the urine than controls do during rest as well as after xercise, is probably due to the differ ent time schedule used. It is l kely that it takes more than 24 h fter exercise before truly basal resting conditions are achieved, since no difference in excretion was found between trained rats and controls 24 h to 48 h after the last period of xercise whereas from 48 h to 72 h after xercise th trained rats excreted less NA than control rats by the same proportion (i.e. 67% of the control value) as that seen immediately after exercise in both studies (66 to 64% of control value) Thus the results suggest that chronically exercised animals require lower degree f overall sympathetic tone in order to maintain homeostasis while at rest as well as during exercise. This is true for human subjects as well both at rest and during and after exercise (Johnson *et al* 1974) A lowered sympathetic tone, as reflected in decreased half life of tissue stores of NA, has been shown to xist both i the heart and in the spleen of trained rats (see Section F) Since the nerves innervating the heart and spleen nd the drenal medullary secretion give rise to only minor fraction of the NA excreted in the urine t rest whereas the major fraction arises from nerve terminals in the vasculature (Bigelow *et al* 1969 Spector *et al* 1972) it is unlikely that these findings on their own could account for the observed diminution of urinary excretion f NA in trained ats. However the increase in the sensitivity to the vasoconstrictor actions of NA found the vascular bed f trained rats (see Section G) could well account for the observed difference in NA xcretion in the urine as trained rats re known to maintain normal blood pressure (Crews and Aldinger 1967 Ljungqvist and Unge 1972).

The possible occurrence in trained rats of increased sensitivity to calorigenic actions of NA (see Chapter 4) might also be contributory factor

Vanillyl mandelic acid xcretion

An increase in the rat of metabolic degradation of released catecholamines in trained ts is a hypothetical mechanism whereby a decreased xcretion of NA and adrenaline in the urine could be explained. In order to xclude this possibility the urinary xcretion of one of the major metabolites,

VMA, was studied. The finding that trained rats excreted significantly less VMA in the urine at rest than the control rats did, is evidence that there is in fact no increased metabolism of catecholamines in the trained rats. Furthermore there was no significant difference in the relative proportion of total CA excretion to VMA excretion in the two groups (npublished observation) Thus, the lower CA excretion in trained rats is not due to increased metabolism but must reflect an overall decrease sympatho-adrenal secretory activity

After cessation f exercise

6 months after cessation of exercise the overall sympathetic activity as monitored by urinary NA excretion, observed in trained rats is no longer significantly different from control values during rest (92% of control excretion) whereas the urinary excretion of NA fter exercise was still significantly lower (84% of control value) The persistence in ex-trained rats of lower urinary excretion of NA after exercise could at least partly be due to the persistence of cardiac hypertrophy in these rats (see Section B) leading perhaps to less sympathetic nervous activity in the hearts of these animals. Although the NA overflowing from the heart is probably minor fraction of the NA excreted in the urine at rest (see above) it probably constitutes more significant fraction of the NA excreted in the urine fter exercise since the NA turnover in the heart of these rats increases 10–17 fold during swimming (see Section F) On the other hand, the unchanged resting excretion of NA suggests that the increased sensitivity to the vasoconstrictor actions of NA seen in trained rats (see Section G) is not permanent but disappears after discontinuation of the regular exercise as is also the case with the increased tissue sensitivity t calorigenic actions of NA in cold-acclimated rats retransferred to a warm environment (Jansky *et al* 1967)

SECTION E EFFECTS OF INTERMITTENT EXERCISE

The effects f intermittent as opposed to daily xercise were studied in previously unpublished experiment.

MATERIALS AND METHODS

Male Sprague—Dawley rats, initially weighting about 170 g were divided in three groups, one served as controls, one was submitted to daily swimming exercise according to the schedule published in paper II with the daily swimming time increased stepwise from 1 to 2 h/day The third group was submitted to swim training three times week only on lternative week days, but was given the same increase in daily swimming time at the same time after the start of the experiment, as the daily exercised group. The total duration of swim training was 15 weeks and the urine collections were performed during the 14th week. Urine was collected during 22 h period firstly fter 24 h of rest and secondly immediately fter 2 h swimming period. Urine collection and CA extraction and determination in organs were performed as described in Chapter 2. Cardiac NA content was assayed according to Chang (1964) and CA content in adrenal glands and urine according to Euler and Lishajko (1961)

RESULTS

The results are summarized in Table 1 and Fig. 1 Whereas intermittent exercise in contrast to daily exercise has no effect on body weight, it does on the other hand produce the same degree of

Table 1 Comparison of the effect of daily and intermittent exercise on organ weight and organ catecholamine content.

Percentage figures given within parenthesis show the increase in catecholamine content as percentage of control value.
Symbols different from control $p < 0.05$ different from control $p < 0.01$ different from control $p < 0.001$ b different from the daily exercised group $p < 0.01$ c, different from the daily exercised group $p < 0.001$

	Controls	Intermittent	Trained
Initial BW (g)	173 ± 2	173 ± 2	175 ± 1
Final BW (g)	427 ± 4	438 ± 4*	407 ± 4
Heart wt (g)	1.28 ± 0.02	1.39 ± 0.02*	1.37 ± 0.02*
Heart ratio g/100g BW	0.298 ± 0.003	0.311 ± 0.003* ,*	0.334 ± 0.006
Adrenal wt (mg)	41 ± 1	46 ± 1	45 ± 1
Heart			
NA µg	0.93 ± 0.03	1 11 ± 0 04 (+ 19%)	1 16 ± 0.04 (+ 25%)
NA µg/g	0 73 ± 0.03	0.80 ± 0.03 (+ 10%)	0.85 ± 0.03* (+ 16%)
Adrenals			
NA µg	6.25 ± 0.58	7.94 ± 0.36*,b (+ 27%)	12.14 ± 1.03 (+ 94%)
Adrenaline µg	35.6 ± 1.7	41.6 ± 0 7 (17%)	42.8 ± 2.4 (+ 20%)
% NA of CA	15 ± 1	16 ± 1	22 ± 1 (+ 47%)

cardiac hypertrophy as daily exercise does, xpressed as increase in absolute heart weight. Hypertrophy of the adrenal glands also occurs in intermittently exercised rats and is f the same magnitude as that seen fter daily exercise The effects on the CA content f the heart and adrenal glands, on the other hand, are not so marked as those which occur after daily exercise. As is the case in daily exercised rats the urinary excretion of both adrenaline and NA after 2 h period f xercise was significantly lower in intermittently exercised rats as compared with controls. When intermittently exercised rats are compared, on the other hand with the group that was exercised daily there is no significant difference between the amounts of NA excreted in the urine after exercise whereas the mount of adrenaline xcreted is even lower in daily exercised rats than it is in the intermittently exercised group ($p < 0.05$)

DISCUSSION

The finding that intermittently exercised rats do not exhibit the decreased body weight found in animals that were exercised daily (for references see Chapter 1 and Section B this Chapter) suggests that the alternate day of rest ensures enough f compensatory increase i food intake on the day of rest to make up for the relative hypophagia on the day of the exercise session (for references see Discussio in Section B) and for the increase in energy xpenditure during the physical exertion.

The observation that intermittently exercised rats display the same degree of cardiac hypertrophy (+6%) as daily xercised rats (+7%) suggests that it is the intensity and duration f exercise sessions, rather than the accumulated total hours of xercise, that determines the degree of cardiac hypertrophy obtained after physical training (see also Discussion in Section B) My findings on body weight and heart weight are seemingly at variance with the results of Leon and Bloor (1968) and Bloor and Leon (1970) who reported that intermittent exercise leads to cardiac hypertrophy in very young rats only not in adult rats, but that it always leads to body weights significantly below control values. This discrepancy is probably due to different experimental procedures. Thus the absence f cardiac hypertrophy in inter mittently xercised rats in their experiment is probably due to less severe training schedule, one h f swimming twice weekly as compared to the 2 h f swimming three times week that the intermittently trained group was submitted to in this experiment Furthermore Leon and Bloor swim their rats in water at temperature f 28–32 C, *as compared to the water temperature of 34–36°C used in our* swim studies, and thereby add an appreciable element f cold stress in thei experiment. This could perhaps explain why they see a reduction in growth rate in their intermittently exercised nimals as well since cold exposure can lead to decreased weight gain (Schwabe *et al* 1938 Heroux and Hart 1954)

The fact that the heart ratios in the intermittently exercised rats were significantly lower than those of the trained rats, is only reflection of their higher body weight (see Discussion in Section B).

Intermittent training also does not differ from daily xercise in the ability to produce adrenal hypertrophy which agai suggests, just as in the heart that as long as the exercise sessions are fairly frequent it is more the work load in the individual session rather than the accumulated hours of work that determines the degree of adaptive adrenal cortical hypertrophy

On the other hand, intermittent xercise does not produce as much increase in the NA content of the adrenal gland as daily xercise does, and although the NA-content in the heart is not significantly lower than after daily xercise the *percentage increase in NA-concentration compared with controls* is nevertheless smaller +10% as compared to +15%. Thus it appears that the mechanisms responsible for the increase in heart and adrenal CA content seen in trained rats, namely enzyme induction and in the case f the heart perhaps also growth of new terminals (see Section C) need daily stimulus to produce maximal increases f transmitter content. This fits with the finding f Leon and Bloor (1968) that intermittent xercise caused rise in capillary/fibre ratio that was only bout 60% f the rise seen in rats that were xercised daily

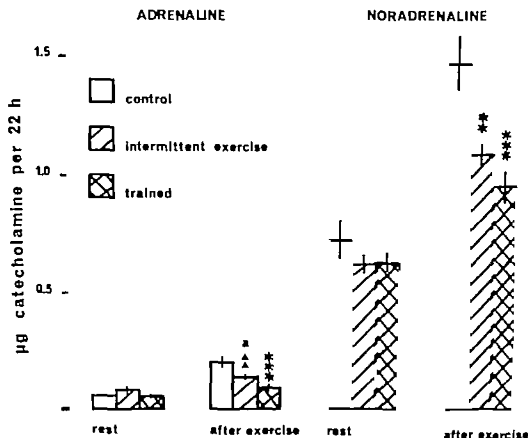


Fig. 1 Comparison f the effect of daily and intermittent training on the excretion of catecholamines in the urine

The values are given as µg catecholamine/22h mean ± S.E

Symbols: open bars control values striped bars values from intermittently exercised rats cross hatched bars values from rats subjected to daily xercise different from control $p < 0.01$ different from control $p < 0.001$ ▲▲ different from control $p < 0.02$ different from the daily exercise group $p < 0.05$

The "resting" urine collections were made 24 h after the last period of exercise. I view f our later finding (paper III) that at least 48 h f rest is probably necessary to reach a truly basal level of sympathetic nervous activity and thereby minimal xcretion of NA in the urine significant difference between intermittently exercised rats and control rats in the resting urinary excretion of NA might therefore have been masked.

However the finding that both the trained and intermittently exercised groups excrete significantly less NA after exercise than control rats do (see Fig. 1) suggests that intermittently exercised rats may share the decrease in overall sympathetic nervous activity found in daily exercised rats both at rest and after exercise (see Sections D and F) possibly as result of increase in peripheral sensitivity to the vasoconstrictor action f NA (see Section G) and perhaps mediated via an increase in the inhibitory activity f noradrenergic neurons i the brain as discussed in Chapters 5 nd 7

Intermittently xercised rats can be expected to have adapted to the emotional stress of the swim training to the same degree as rats trained by daily xercise. However the amount of adrenal ne creted in the urine f intermittently exercised rats after exercise was significantly higher than that of daily exercised rats ($p < 0.05$) although it was still significantly lower than that of controls ($p < 0.02$). This observation suggests that adaptation to th emotional stress f swimming is not the only factor contributing to the diminution of exercise-induced rise in urinary xcretion of adrenaline seen in trained rats, but that there is further factor presumably an adaptive response to repeated physical xertion itself Furthermore it appears that the latter adaptive response is only fully expressed in animals subjected to daily exercise sessions, as opposed to sessions on alternate days. This adaptation could involve cardiovascular and/or "metabolic" e.g. substrate-mobil zing, actions f catecholamines and it is noteworthy in this context that intermittently exercised rats do not achieve the significant increase in the proportion f adrenal CA content that is comprised by NA that is found in daily exercised rats.

SECTION F TURNOVER OF CATECHOLAMINES IN VARIOUS ORGANS

(Papers II, III and unpublished observations)

During steady state conditions the CA content in the neuron is maintained because the rates of synthesis and reuptake are equal to the rates f release and intraneuronal degradation. Thus turnover of the transmitter is equivalent t release plus intraneuronal degradation less reuptak and equals the rate of synthesis during steady state conditions The rate f NA turnover is proportional to the amount f nervous activity in the peripheral sympathetic neuron and turnover is virtually abolished by decentralizatio (Oliverio and Stjarne 1965 Alousi and Weiner 1966 Gordon et al 1966[a] Sharpe, 1967) Sedvall et al 1968 Swedin, 1970) Since transmitter concentrations in adrenergic neurons are acutely altered only during xperimental conditions that lead to very marked and/or prolonged increases in nervous activity (see Section C for references) it essential to study the turnover of NA, rather than the endogenous levels, in order to detect more moderate alterations in nervous activity Various methods have been designed to study turnover rate but undoubtedly the most physiological ones are those that avoid any pharmacological manipulations by achieving radioactive labelling of transmitter stores, either by administration of labelled NA or of labelled precursor The turnover rat can then be estimated by pplying steady state kinetics to the disappearance of the labelled transmitter (see Montanari et al 1963)

After administration f l– H NA the disappearance of the labelled substance follows an ponential time course from 1 h onwards in the rat heart (Iversen et al 1971). The rat f d

of labelled DA and of NA, formed from labelled tyrosine, has been shown to be xponential in the rat brain from 2 h or earlier after injection of the labelled precursor (Persson 1969 Nybäck, 1971) and in the rat heart from 1 h onwards (Persson, 1969)

In order to obtain an est mate of sympathetic nervous activity in organs of chronically exercised rats the turnover f NA has been studied using both exogenous l–^{3}H–NA and labelling f endogenous NA by L–^{14}C-tyrosine

RESULTS

The estimated half-lives of the transmitter stores n various organs are given in Table 2 and Fig. 2, based on some previously unpublished results as well as data from papers II and III The data from paper II are converted from specific activity i.e. c.p.m./µg NA, to the total c.p.m. f ^{3}H–NA per organ since the endogenous NA levels were altered during one of the experiments.

Brain

The turnover rates of DA are the same in trained rats and control rats both at rest and after xercise and the turnover rate is not changed by exercise in either group. The half-lives of NA are also the same n both groups, but since the trained rats have significantly more NA in their brains (see Section C) the actual amount of NA turning over per unit of time is higher in trained rats. Using the formula f Brodie *et al* (1966) (total turnover equals steady state concentration times the rate constant [$\log_e$ base] for disappearance) and correcting the steady state concentration for the recovery the assay method, ma imum turnover rate of 0.088 µg NA/g/h is calculated for trained rats, compared with 0.070 µg NA/g/h the controls, representing an ncrease f 26%. (Due to a misprint in Table 1 in Paper V where the NA concentration in the brains of trained rats was given as 0.37 µg/g, rather than the correct figure of 0.35 µg/g, an erroneous turnover figure f 0.093 µg/g/h for the trained rats was unfortunately given in paper V.)

There appears to be an increase in NA-turnove in the bra wh le the rats are swimming, compared with when they are at rest, both in controls (+42%) and in trained rats (+31%) however only in the trained group is the slope of the regression line of p.m. f ^{14}C–NA versus time signif cantly smaller ($p < 0.02$) at rest than during swimming (previously unpublished it must be emphasized that the turnover rates f CA in the brains of swimming rats should only be compared with the rest ng values because in this xperiment the tra ned rats, that were swimming in separat bath from the control rats, displayed much more active swimming than control rats did nd thus these groups are not strictly comparable with each ther)

Heart

The half-life f NA in the hearts of trained rats is sign ficantly longer than in the hearts f control rats. This was shown ndependently by using either exogenous l–^{3}H–NA (paper II) or L–^{1} C-tyrosine (paper V) for labelling of transmitter stores. Using data from paper II th total turnover of the transmitter store can be estimated to be 0.041 µg NA/g heart /h in the controls but only 0.010 µg NA/g heart/h in the trained rats. I fact in both tudies the slopes f the regression lines f labelled NA versus time re not significantly diff rent from zero in the trained groups and thus one can say that there is no significant turnover f transmitter detectable over 12 h period, in the heart f resting trained rats. During swimming there is marked increase in cardiac NA turnover in both groups, but the fractional turnover rate is st ll significantly lower in trained rats ($p < 0.01$) with $t_{½}$ of 2.8 h compared with 1 4 h i the controls Furthermore, the rate of NA turnover is significantly lower in the hearts of

Table 2. The turnover rate of dopamine and noradrenaline in brain, heart and spleen of trained and untrained rats, at rest and during swimming.

Symbols: $t_{1/2}$ = half-life, k = rate constant, S.D. standard deviation

	Slope (k) ± S.D.	$t_{1/2}$ (h)		k ≠ 0	Method
BRAIN NA					
Rest					
Controls	–0.064 ± 0.012	6.5		$p < 0.01$	L–^{14}C-tyrosine
Trained	–0.065 ± 0.006	6.6		$p < 0.001$	
Swimming					
Controls	–0.077 ± 0.013	3.9		$p < 0.001$	
Trained	–0.072 ± 0.012	4.2		$p < 0.001$	
BRAIN DA					
Rest					
Controls	–0.082 ± 0.006	3.7		$p < 0.001$	L–^{14}C-tyrosine
Trained	–0.090 ± 0.006	3.3		$p < 0.001$	
Swimming					
Controls	–0.093 ± 0.014	3.2		$p < 0.001$	
Trained	–0.092 ± 0.012	3.3		$p < 0.001$	
HEART NA					
Rest					
Controls	–0.016 ± 0.004	18.6	$p < 0.001$	$p < 0.01$	L–^{3}H–NA
Trained	–0.003 ± 0.005	104.0		n.s.	
Controls	–0.037 ± 0.017	8.0	$p < 0.001$	$p < 0.005$	L–^{14}C-tyrosine
Trained	–0.004 ± 0.012	80.0		n.s.	
Swimming					
Controls	–0.214 ± 0.063	1.4	$p < 0.01$	$p < 0.01$	L– H–NA
Trained	–0.118 ± 0.018	2.6		$p < 0.005$	
SPLEEN NA					
Rest					
Controls	–0.064 ± 0.020	5.5	$p < 0.001$	$p < 0.001$	L–^{14}C-tyrosine
Trained	+0.00006 ± 0.017	∞		n.s.	"

Note that all the numerical values of k in this table have been derived from regression lines where the Y-values have been expressed in $\log_e$, thus those values originating from paper V have been converted from $\log_{10}$-based values to $\log_e$-based values.

trained rats compared with controls also during intermittent swim exercise (see Fig. 2 data from paper II)

Spleen

I this organ as well the fractional turnover rate of NA was significantly lower in trained rats than in control rats. The slope of the disappearance of the radioactively labelled NA versus time was not significantly different from zero in the spleen f the trained rats, and the estimated half-life was "infinite

DISCUSSION

Turnover of DA and NA in the brain

The calculated half-lives of DA and NA reported in paper V are in good agreement with previously published half-lives estimated by different methods (Iversen and Glowinski 1966 Taylor and Laverty 1969 Persson 1969 and Nybeck, 1971) The half-lives of both NA and DA in the brain, as estimated by disappearance of ^{14}C—CA after ^{14}C-tyrosine administration are not changed by chronic exercise. However since both the concentratio nd the total content of NA, but not those of DA, re signif cantly increased brains of the chronically exercised rats, the total mount of NA turning over per unit time is increased in the brains f the trained animals at rest. This finding is very unlikely to be due to any persisting effect of the acute physical stress of the last swimming period since the turnover study using ^{14}C-tyrosine was preceded by 48 h of rest and since the turnover rates f NA in the two peripheral organs studied were in contrast to that in the bra n, markedly lower in trained than in control rats. Thus the bserved increase in total NA turnover in the bra f tra ned rats is unl kely to be an xperimental rtefact.

The increased NA content in the brain of chronically exercised rats might be an adaptive response to daily bouts f increased nervous activity in noradrenergic neurons, as discussed Section C. It is well known that during forced running f untrained rats there is an ncreased turnover f NA in whole bra and brain stem (Gordon *et al* 1966[b]) as well as hypothalamus (Stone 1971 1973) Heat has been found to increase the turnover f NA whole brain (Corrodi *et al* 1967) as well as the hypothalamus (Simmonds, 1969) and forced running can easily induce hyperthermia. However as shown Table 2, an increase in turnover rate f NA in whole brain occurs during swimming just as it does with forced running Since this increase occurs in trained rats which are emotionally adapted to swimming and is f the same magnitude as in controls, it appears that th increase in turnover rate of NA in the brain may be response to muscular work rather than to more non-specific factors such as emotional stress. In this context the possibil ty must also be considered that, during xercise there is increased activity in noradrenergic pathways that might participat in the hypothalamic regulation of endocrine functions since t is known that both ACTH (Frenkl *et al* 1968 1969) and growth hormone (Hunter *et al* 1965 Sutton *et al* 1969 Hartley *et al* 1972) levels in plasma are elevated by xercise In fact changes in the turnover f NA in noradrenergic neurons in the hypothalamus are perhaps more l kely to account for the observed increase in total brain turnover f NA than for example noradrenergic neurons in the lower brain stem. This is because th activity of some f the latter pathways is assumed to stand in an inverse relationship to peripheral sympathetic nervous activity (see Chapters 5 and 7) which f course, is markedly increased during xercise.

It is noteworthy that 3 h of moderate swimming activity does not increase he turnover ate of DA in the brain ither in controls or in chronically ercised rats (Table 2) Bliss and Ailio (1971) have reported that swimming in wa er at 37 C leads t increased levels f homovanillic acid (HVA)

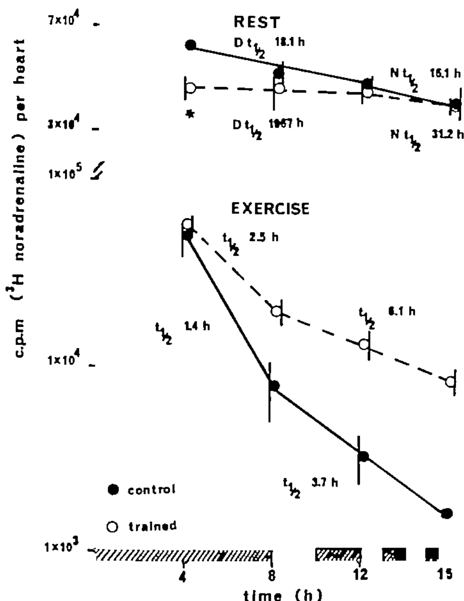


Fig. 2. The effect of chronic exercise on cardiac turnover of noradrenaline at rest and during exercise
Symbols as in figure.

The values given are means ± S.E. note that the logarithmic scales on the y-axis are different in the two parts of the figure. $Dt_{½}$ denotes half-life during day time (the period 4h—12h) $Nt_{½}$ the half-life during night time (the period 12h—16h) denotes different from control $p < 0.05$. Data from paper III but expressed as c.p.m. ($l-{}^{3}H-NA$) per heart rather than as previously c.p.m./µg NA. The shaded areas along the -axis shows the periods during which the two groups of rats were subjected to swimming for the determination of cardiac NA turnover during continuous (0—8h) and intermittent (8—16h) exercise

I the brain of mice but this need not be at variance with the above findings DA turnover since Hutchins *et al* (1975) have shown that the proportion of DA metabol tes that is constituted by HVA, and that constituted by 3 4–dihydroxyphenylacetic acid (DOPAC) is xceedingly sensitive to emotional stress and to factors such as body temperature so that it is possible to see an increase in the levels of one of the metabolites without having an increased turnover of DA.

NA turnover in the heart

The significantly decreased turnover f both endogenous NA (formed from ^{14}C tyrosine) nd exogenous ^{3}H–NA in the hearts of chronically exercised rats suggests that the flow of impulses in the cardiac sympathetic nerves is decreased in tra ned rats. This is so because other xplanations for the changed turnover rate can almost certainly be excluded Firstly the decrease NA turnover is not l kely to be accounted fo by superefficient, almost 100%, reuptake of released NA since the uptake and retention of xogenous NA into the sympathetic erves the hearts of chronically exercised animals is in fact significantly lower than that seen I ntrained rats (paper II Fig. 2 this Chapter); this fact in itself might indicate that the cardiac sympathetic nervous activity is lower in the trained rats since it has been claimed that increases in impulse flow lead to higher degree of reuptake (Häggendal and Malmfors, 1969) Secondly an effect on turnover rate of the ts seen these ex periments cannot be expla ned by decrease in intraneuronal metabolism alone, since this contributes quantitatively very small proportion of the total transmitter turnover as seen by the insign ficant NA turnover decentralized organs (Sedvall *et al* 1968 Swedin 1970) Thus it appears that chronically xercised rats display genuine and marked decrease the activity of cardiac sympathetic nerves.

There have been several suggestions that newly synthesized NA may be preferentially released and that there may be different pools f NA with nerve endings (Trendelenburg, 1961 Kopi *et al* 1966 Sedvall *et al* 1968 Thierry *et al* 1971 Stjarne, 1971) An alteration the distribution f NA between the hypothetical "functional/labile and "storage" pools in cardiac sympathetic nerves trained rats, so that newly synthesized ^{14}C–NA is used for transmissio for shorter period than controls and thus becomes stored in a "passive pool earlier could theoretically lead to an apparent decrease in turnover rate since the period studied begins 2 h after the injection of the labelled precursor Such mechanism xtremely unl kely t account for the non-detectable t rnover the hearts f trained rats found in the ^{14}C-tyrosine study since the turnover study using xogenous ^{3}H–NA showed that the same non-detectable turnover f cardiac NA occurred in chronically exercised rats during day time. If xogenous NA is distributed unequally within these hypothetical pools it is predominantly labelling the rapidly xchanging "pool" in the heart as discussed by Lammer and Saller (1974) Furthermore the same non-detectable turnover f cardiac NA in trained rat is found in the 0–4 h nterval when the turnover rate is estimated by synthesis inhibition by α–methyl paratyrosine method which is not f course influenced by any unequal distr bution within various pools (see Chapter 5) Lastly these findings are also corroborated by the decreased urinary xcretion of NA in the trained rats (see Section D)

It may seem improbable that at rest there should be no significant sympathetic ervous activity at all in the hearts of trained rats but t should be remembered that nocturnal species such as the rat spends most f the day sleeping and thus in true state f maximal rest. Lammer and Saller (1973 1974) have shown that during the day the turnover f NA is sign ficantly lower than during the ight the period of most activity Indeed f the data from the ^{3}H–NA xperiment (paper II) is divided in a day" part, consisting of the 4–12 h interval and night" part consist ng of the 12–18 h interval one can discern the same tendency in that tudy Whil the $t_{½}$ of cardiac NA in control rats is 18 1 h during "day" it is 16 1 h during ight (n.s. day value), whereas in the trained ats he $t_{½}$ during "day" is 1967 h (82 days d fferent f m control $p < 0.001$) compared with $t_{½}$ of 31.2 h during

night (n.s. v. day value) (see Fig. 2).

Relative bradycardia trained rats compared with control rats persists with exercise-induced elevation of heart rate (Ekblom *et al.* 1973) and similarly the turnover rate f cardiac NA remains lower than that of control rats both during continuous and intermittent exercise (see Fig. 2). Thus the half life of cardiac NA during continuous swimming is 2.5 h in trained rats as compared with half-lif of only 1.4 h in the controls ($p < 0.01$) and during intermittent swimming the half-lives wer 6.1 h and 3.7 h for chronically xercised rats and controls, respectively ($p < 0.05$).

It seems l kely that at least part of the reason for the decrease in sympathetic tone in trained animals is the cardiac hypertrophy associated with larger stroke volume (references see Chapter 1 and Section B this Chapter) which would lead to a lower heart rate being necessary to maintai any given cardiac output. Howeve it is possible, considering the changes in NA content and total transmitter turnover that occurs in the brain of trained rats, that the brain is actively involved in maintaining this low sympathetic tone in the heart spleen and probably in the vasculature (see Sections D and F) as opposed to maintaining it only o the basis of unchanged cardiovascular reflexes. The finding that trained rats have normal blood pressure (Crews and Aldinger 1967 Ljungqvist and Unge, 1973) suggests that the total peripheral resistance is more or less unaltered and might thus indicate that the role of the brain is passive one. Whether or not the brain plays an active or passive role in instigating decreased sympathetic tone in chronically exercised animals the maintenance of this decreased sympathetic nervous activity is however probably inversely related to, and dependent upon, the nervous activity of some noradrenergic pathway(s) in the brain as discussed in Chapters 6 and 7

Noradrenaline turnover in the spleen

Vascular adjustment during heavy xercise involves an increased sympathetic nervous activity in the spleen (Gordon *et al.* 1966[b] Sheldon *et al.* 1975) leading to vasoconstriction the spleen which may function as reservoir of blood in some species. That this is true in rats as well is shown by 13% decrease in spleen weight that occurs fter 3 h period of swimming (unpublished observation) I view of this, *and of the increased sensitivity to the vasoconstrictor action f NA found chronically* exercised rats (see Section G) it is hardly surprising that the spleen in trained rats shows an adaptive decrease in sympathetic tone just as the heart does. Furthermore this decrease in sympathetic tone in chronically xercised rats probably occurs generally in th vasculature as well (see discussion in Section D)

CONCLUSION

In summary therefore chronically xercised rat xhibit reciprocal changes between activity in central and *many peripheral noradrenergic neurons there is an increase in total transmitter turnover in* the brain, presumably reflecting increased nervous activity and decrease in NA turnover in many peripheral organs, notably the heart, the spleen and probably the vasculature

SECTION G EFFECT OF CHRONIC EXERCISE ON EFFECTOR ORGAN RESPONSE TO CATECHOLAMINES A STUDY ON PERFUSED HINDBODY AND ISOLATED HEART

(Paper IV and unpublished data)

Chronically xercised rats xhibit decreased urinary xcretion of NA (papers II, III Section D) and decreased urnover rate f NA in organs such as heart and spleen (papers II III, Section F) The

possibil ty that these adaptive changes activity of the sympathetic nervous system are reflex-mediated following an increased sensitivity of effector tissues to the actions of NA has been studied in two important effector organs namely the vascular bed and the heart. The vascula bed was studied in the perfused hindbody preparation as described by Folkow *et al* (1970) with minor modifications, and the heart in the isolated heart preparation of Langendorff (see Chapter 2 for details)

RESULTS (see Table 3 nd Figs. 3 and 4)

Vascular bed

Dose-response curves for the vasoconstrictor action of NA were obtained by plotting the steady-state pressure level for each stepwise rise in NA-concentration in the perfusion fluid as pressure versus $\log_{10}$ dose NA. A dose-response curve was fitted by eye to the xper mental points and the M_{50} (see below) and the 120-dose" (see below) for each individual curve was determ ned as the base for statistical comparison between the two groups. In Fig. 3 the individual points re the means (+ S.E.M) of all individual pressure responses recorded at the various dose levels for each group.

In a first series of experiments the NA solutio used for the infusion had ascorbic acid added to protect the NA from xidation during the course of the exper ment this was however not sufficient and in the series on which the dose-response curve seen in Fig. 3 is based 2 µM disodium ethylenediamine tetra-acetate (EDTA) was present as well and this gave satisfactory protection against oxidation of the NA. As far as the data on perfusion pressure at maximal dilatation, maximal pressure response and M_{50}-pressure (see below for definition) are concerned the two series gave almost identical results and thus in these respects the results from the two series were pooled to give the data in the first part f Table 3

M_{50}-dose is the dose t which 50% of the maximal pressure response is obtained. This is not the same as the ED_{50}-dose as discussed by Folkow *et al* (1970) since it corresponds to 80% of the maximal increase in flow resistance and not to 50% f the maximal contraction f the ndividual smooth muscle cell, as the increasing wall/lumen ratio of the constricting vessels more and more potentiates the reduction in size f the lumen resulting from a given degree of smooth muscle shortening. Thus the true ED_{50}-dose is lower than the M_{50}-dose (see Folkow *et al* 1970) Since th maximal pressure response in the vascular bed of trained rats is higher than that seen n control rats further entity the "120-dose" has been ntroduced to enable direct comparison f the physiologically relevant sensit vity of vascular smooth muscle The "120-dose" is defined as the concentratio f NA in the perfusio fluid that maintains perfusio pressure of 120 mm Hg This pressure level was rbitrarily chosen as it is roughly on the middle f the steeply-rising, linear part of the dose-response curves as well as being within the physiological range

As can be seen from Table 3 nd Fig 3 ther was no change in th perfusion pressure at maximal dilatation or in the slope of the linear part of the dose-response curve, in perfused hindbodies of chronically xercised rats compared with those of control rats. O the ther hand there was a highly significant 14% increase of the maximal pressure response with consequent increase in ma imal per fusion pressur obtained in the vascular bed of chronically exercised rats Furthermore the dose-response curve to NA in perfused hindbodies of chronically exercised rats shows parallel shift t the left when compared with controls, resulting in a highly sign ficantly lower M_{50}-dose and mean 120-dose in the trained group. As result f this parallel shift, the vascular bed f chronically exercised rats needs 38% lower NA concentration than that required by the vascular bed f controls in order to maintain given perfusion pressure in the range of 80—160 mm Hg thus he sensitivity to the vasoconstrictor actions of NA is markedly increased in trained rats

Table 3. The effect of regular exercise on the pressure response and the sensitivity to the vasoconstrictor action of noradrenaline in the perfused hindbody of th rat and on chronotropic and inotropic responses to noradrenaline in the isolated rat heart.

Values given are means ± S.E. with the exception of the slopes of the linear part f the dose-response curve which are given as means ± S.D

Symbols: C = control T trained, k slope (log_{10} base) f the dose response curve.

Perfused hindbody

	Controls (n = 14)	Trained (n 14)	p-value C v. T
Pressure at maximal dilatation (mm Hg)	26 ± 1	26 ± 2	n.s.
Maximal pressure (mm Hg)	202 ± 3	226 ± 3	$p < 0.001$
Maximal pressure response (mm Hg)	176 ± 3	200 ± 3	$p < 0.001$
M_{50}-pressure (mm Hg)	114 ± 2	124 ± 6	n.s.
	Controls (n 7)	Trained (n 8)	
Slope (k-value)	189 ± 16	196 ± 14	n.s.
M_{50}-dose (µM NA/ml)	0.96 ± 0.04	0.68 ± 0.06	$p < 0.001$
120-dose (µM NA/ml)	1.07 ± 0.06	0.66 ± 0.04	$p < 0.001$

Langendorff heart

	Controls	Trained	p-value C v. T
Spontaneous heart rate (beats/min)	277 ± 14 (n 9)	261 ± 9 (n 8)	n.s.
Maximum heart rate (beats/min)	403 ± 19 (n 7)	395 ± 14 (n 6)	n.s.
Maximum heart rate increase (beats/min)	130 ± 16 (n 7)	119 ± 18 (n 6)	n.s.
ED_{50} dose chronotropic response (µM NA/ml for 10 sec)	3.10 ± 0.12 (n 6)	3.94 ± 0.66 (n 4)	n.s.
slope (k-value) chronotropic	42 ± 18	60 ± 16	n.s.
M_{50} dose inotropic response (µM NA/ml for 10 sec)	1.96 ± 0.33 (n 4)	1.97 ± 0.48 (n 3)	n.s.

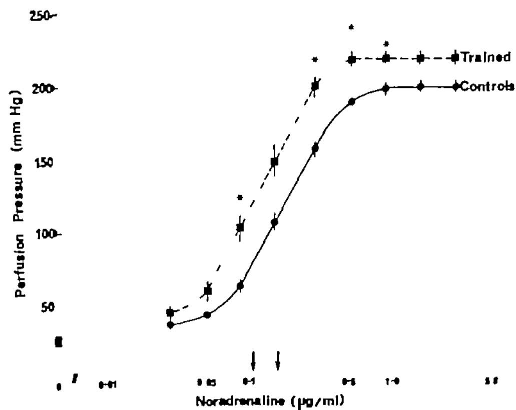


Fig. 3 Dose-response curve to noradrenaline in the perfused hindbody of trained and control rats. Symbols see figure.

The values given are means and the vertical bars represent the S E (control 7 trained rats 8) denotes different from control $p < 0.06$ different from controls $p < 0.01$ different from controls, $p < 0.001$ ✦✦ denotes different from control $p < 0.02$. N te that when larger number of experiments are pooled, the difference in maximal pressure obtained in the two groups is highly sign i cantly different (see Table 3) The two arrows indicate the M e-dose which is also highly significantly ferent in the two groups (see Table 3)

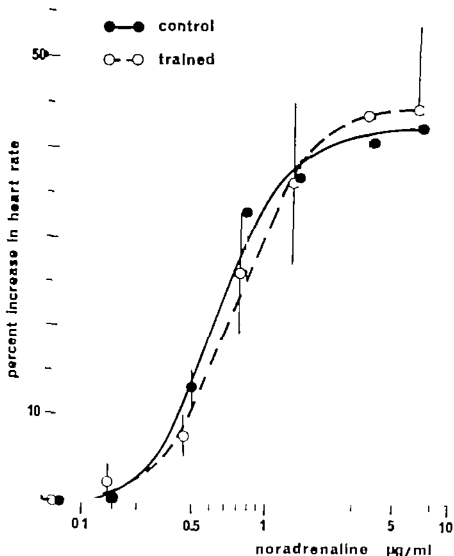


Fig. 4 Chronotropic response to noradrenaline in the isolated rat heart perfused by Langendorf procedur

The values given are means ± S.E. (Controls 5 trained 4). Curves are f tted by eye to the experimental points and the calculated mean ED dose. Note that the doses employed in the two experimental groups are not identical (due t the 7% difference in perfusion rate) which largely accounts for the difference between the experimental points f the two groups on the steep-rising par of the dose-response curve. Furthermore note that noradrenaline was given in the concentrations indicated as 10 second stat infusion only.

Heart

The intrinsic heart rate of isolated hearts perfused by the method of Langendorff showed a considerable individual variation in both groups but the means were not different (Table 3). The maximal heart rate also varied considerably between individual hearts, but there was no systematic d fference between hearts of chronically exercised rats and those of control rats (Table 3)

There was no significant difference in the ED_{50}-dose for the chronotropic response t NA between the two groups (Table 3) The fact that th mean value n the trained group is a bit higher is because a single observation had a considerably higher value (5.80 μM/ml) tha the rest Preliminary observations (unpublished) have also suggested that there is no difference between hearts from control rats and trained rats the chronotropic response to isoprenaline. I order to mi imize the influence f the large individual variations in the intrinsic heart rate leading to a wide scatter of heart rates obtained in response to a given dose, the composite dose-response curve of the chronotropic response to NA (Fig 4) is plotted as the % increase of intrinsic heart rate versus log dose f NA rather than as bsolute heart rates this did not, of course alter the relations between the results in the two groups, i.e in the actual configuration or slope of the dose-response curves. As can be seen from Fig 4 the slopes of the dose-response curves, as fitted by eye to the experimental observations, re about the same for the hearts of both groups f rats. This is also conf rmed by a least square analysis of the observations on the linear part of the two dose-response curves. (k—values ± S.D controls 42 ± 18 tra ned 50 ± 16). Furthermore there is no significant lateral shift of the dose-response curve of the trained group.

The main object was to study th chronotropic responses of the isolated heart. It was therefore decided to record the contractions of the heart via balloon inserted into the left ventricle and inflated *in situ* the pressure in the balloon being monitored by pressure transducer as the isolated rat heart is xtremely sensitive to trauma and procedures such as connecting straingauge to the cardiac muscle (which gives better record f inotropic responses) leads t such a high incidence of ventricular arrhythmias that it is mpossible to study chronotropic responses. Intracardiac balloon pressure is bviously an ndirect nd less than ideal way of recording the contractile force However in the experiments where the balloon position remained stable throughout the xperiment, clear dose-response curve could be obtained by plotting the % increase in peak intra-balloon pressure versus log_{10} dose of NA. This sort f curve cannot, f course be used to obta a true ED_{50}-dose but should still be valid for comparisons f relative sensitivity Using th data in the xperiments with stable balloon-position the mean M_5 -dose (50% of maximal response) for the inotropic response to NA was found t be practically identical the two groups (Table 3) Furthermore prel minary observations (unpublished) have suggested that there is no difference between hearts from tra ned rats and from controls in the sensitivity to the inotropic ction f isoprenaline.

I summary there appears to be no sign ficant change in sensitivity t the chronotropic and inotropic actions of NA in the hearts of chronically xercised rats.

DISCUSSION

Isolated heart

The heart l k any other sympathetically innervated tissue develops prejunctional denervation supersensitivity due to removal of neuronal uptak of NA, but t is controversial whethe any post junctional supersensitivity (of the decentralisation-type) occurs Dempsey and Cooper (1968) claim that surgically denervated hearts develop prejunctional denervation supersensitivity only and Haeusler *et al* (1969) suggest that postjunctional supersensitivity is lso absent after chemical sympathectomy of the heart by 6-hydroxydopamine treatment On he ther hand, Westfall and Fleming (1968) claim

that pretreatment with reserpine leads to post-junctional type f supersensitivity which is characterised by partial depolarisation of atrial cardiac muscle cells (Fleming *et al* 1975) and which they suggest is equivalent to decentralisation supersensitivity. These authors were however only ble to demonstrate these changes with some bath media such as Locke's solution but not with a modified Krebs–Henseleit solution. Furthermore it cannot be excluded that the effects observed by Fleming and co-authors are due to non-specific direct pharmacological effects of the reserpine treatment on the cardiac muscle cell rather than a decentralisation type of post-junctional supersensitivity. I summary therefore, there are considerable doubts as to whether the heart is normally capable of developing a postjunctional type of supersensitivity and thus it is not surprising that chronic physical exercise failed to lter the sensitivity of isolated hearts to NA.

The claim f Crews and Aldinger (1967) that there was decreased sensitivity to the inotropic actions of adrenaline in hearts of trained rats can probably be disregarded as it was based not on a proper dose-response curve but on the response to   ngle i. dose, and was not supported by any figures or statistics. Likewise the claim of Pavlik and Frankl (1975) that trained human subjects showed smaller blood pressure response to NA, but an unchanged blood pressure and heart rate response to adrenaline is impossible to evaluate as agai the claim is based only on response to single i. dose, without proper dose-response curve. This is not valid approach since the actual concentration of the drug reaching the organ studied is dependent among other factors on body weight (not given) the percentage of body weight made up by water (not known likely t be different in trained and untrained subjects, see Section B) and in particular th blood volume (known to be increased in trained subjects, see Sjostrand, 1955). Furthermore the response studied is summation of the effects of unknown concentrations of the injected catecholamine with circulating levels f catecholamines (likely to be different in trained and untrained subjects, see Section D) and with the activity of the autonomic nerves (also l kely to be d fferent, see Section F)

It is probably fair t summarise, therefore that any valid conclusion about possible lterations f receptor sensitivity in trained animals must be based on studies on isolated organs.

Sutton *et al* (1967) Lin and Horvath (1972) and Ekblom *et al* (1973) all interpreted the lower heart rate found in chronically exercised humans and rats after simultaneous treatment with atropine and propranolol as being due to lower intrinsic heart rate in the trained individuals. This finding, however is more likely t be due to incomplete β–blockade as the xercise-induced increase in heart rate was not abolished either in the study of Sutton *et al* (1967) or in that of Ekblom *et al* (1973). In the case of Lin and Horvath's study (1972) the dose of propranolol given to the rats (8 mg/kg BW i.p.) is certainly not enough to block very substantial increase in heart rate caused by the increased cardiac sympathetic nervous activity induced simply by handling the rat (unpublished observation). Furthermore in my experiments the ntr nsic heart rat f isolated Langendorff hearts (see Table 3) as well as the heart rate in rats given atropine plus the ganglion blocker chlorisondamine (unpublished observation) is the same in controls nd chronically xercised rats. Using isolated atria from rat hearts Bolter *et al* (1973) reported slower intrinsic rate in the atria from trained rats. There are at least two factors that could xplain the discrepancy with my findings, firstly they studied their atria in an organ bath at non-physiological temperature only 30 C, secondly the isolated atrium is undoubtedly more traumatized preparation than Langendorff heart, particularly when as in the study f Bolter and co-workers, the atria are mounted under tension, and is possible that the slower rate f the atria of the trained rats is due t release of acetylcholine from the atrial tissue since trained rats have considerably increased acetylcholine content in the tria (Herrlich *et al* 1960).

Some authors have suggested that the maximal heart rate might be somewhat lower in trained humans (Ekblom *t al* 1973 Bronson *et al* 1976) however these authors simply studied the heart ate at the maximally tolerated work load, nd no figures were given to prove that the heart rat had actually reached a plateau where no further heart rate increase was possible. In my own study there

was no suggestion of a significant difference between th maximal heart rate in isolated hearts from trained and control rats.

Vascular bed

The perfused hindbody preparation as described by Folkow *et al* (1970) contains a vascular bed that consists largely of the normal resistance vessels in the body Therefore lterations in the sensitivity to NA of this preparation represent changes with a direct physiological relevance for regulation of the blood pressure The degree of increased sensitivity found i trained rats is such that 39% lower concentratio of NA than in control rats is required to produce the same increase in perfusion pressure this is remarkably paralleled by the finding that trained rats at rest excrete 33% less NA than control rats (see Section D) Thus, the reduced release of NA from vascular erves in trained rats should be just adequate to maintain normal blood pressure as has indeed been f und xperimentally (Crews and Aldinger 1967 Ljungqvist and Unge, 1972)

Altered dose-response curves to NA in vascular preparations such as the perfused hindbody can be caused by a variety f changes for xample 1) Hypertrophy f th smooth muscl layer i th tunica media of the vessel wall 2) Increased contractility per unit f contractile tissue, 3) Changes in the size of the vascular bed 4) Altered uptake of NA into sympathetic nerves. 5) Altered extraneuronal uptake of NA. 6) True change in the sensitivity f smooth muscle to vasoconstrictor properties of NA mediated either by an alteratio in the properties f the α–receptors the β–receptors or both. These various possibilities will now be discussed in turn to see which is the most likely to account for the changes found in chronically exercised rats.

It is known that hypertrophy of the media in the arterial vessel wall leads to more steeply rising dose-response curve as seen for instance in spontaneously hypertensive rats (Folkow *et al* 1970) This occurs irrespective of whether or not the hypertrophy of the media encroaches on the lumen at maximal dilatation because the same degree of shortening f the individual smooth muscle cell will lead to a more pronounced decrease in lumen size when the wall/lume ratio is higher than normal (see Folkow *et al* 1968) However when the hypertrophied muscle does ncroach on the lumen at maximal dilatation the perfusion pressure at maximal dilatatio is increased, as occurs in spontaneously hypertensive rats (Folkow *et al* 1970) We can conclude that hypertrophy f the media has not occurred in the trained rats for two reasons. Firstly at a identical perfusion rate the perfusion pressure t maximal dilatation was the same as controls, and consequently there can be no hypertrophy of the vessel wall that encroaches upon the lumen at maximal dilatation. Secondly the slopes of the dose-response curves were the same in both groups.

An increased bil ty to develop active tension per unit f contractile tissue in blood vessel f unaltered design would according t Folkow *et al* (1970) increase both the slope of the curve and the maximal pressor response leading to an unchanged M_{50}-dose. Thus such hypothetical mechanism cannot xplain the parallel shift in the dose-response curve and the lowered M_{50}-dose found in the trained rats.

A decrease size f the vascular bed would lead t an ncreased perfusion pressure t maximal dilatation and steeper slope as the flow per resistance vessel would be higher and neither of these changes are observed here

Blocking neuronal uptak of NA (uptake$_1$) not only sh fts the dose-response curves to l–NA t the left but also decreases the slope of the curve (see eview by Trendelenburg, 1972). Therefore diminished neuronal uptake of NA seems unlikely t be the cause f the observed shif in dose-response curves in chronically xercised rats, since the slopes of the dose-response curves the two xperimental groups are virtually identical

The presence of ster ids in organ bath or perfusing medium has been shown t potentiat the

action of catecholamines by impairing extraneuronal uptake (uptake$_2$) of NA (Kalsner 1969; Iversen and Salt 1970). In this experiment however the perfusion medium contained no steroids and, furthermore, it might be expected that an impaired uptake$_2$ would result in a less steep dose-response curve just as seen with impaired uptake$_1$ (see above).

It therefore appears that there is only one satisfactory explanation for the parallel shift in dose-response curve found in trained rats, namely that there has been a true increase in vascular smooth muscle sensitivity to the vasoconstrictor action of NA. As NA is not only a potent α-agonist but also a β_2-agonist, albeit a rather weak one (Furchgott, 1972), increased vasoconstrictor response could be due either to a decreased sensitivity to β_2-actions or an increased sensitivity to α-agonist actions.

Some evidence that a decreased sensitivity of vascular β_2-receptors can occur in some experimental conditions is provided by the work of Le Blanc *et al.* (1972) who have shown that in rats treated chronically with isoprenaline the acute fall in blood pressure following a dose of isoprenaline is much reduced or even abolished. It is unfortunate that these authors did not publish a proper dose-response curve. Their experimental conditions are similar to the present study in so far as daily exercise provides a daily exposure of the blood vessels to high concentrations of NA, which could conceivably have the same effect on the β_2-receptor of vascular smooth muscle as daily administration of isoprenaline. Furthermore Speiser and Weinstock (1974) suggest that isolation of animals, which leads to decrease in sympathico-adrenal activity (Welch and Welch, 1968, 1969) gives rise to an increased sensitivity of vascular β_2-receptors to both isoprenaline and NA, resulting in decreased pressor responses to NA. In the experiments of Speiser and Weinstock (1974) low doses of NA not infrequently actually led to a fall in blood pressure in isolated, but not in group-housed, rats. This observation suggests that in the isolated rat the threshold for the β_2-agonist actions of NA is lower than the threshold for α-agonist action. Normally this difference in threshold sensitivity is only revealed when adrenaline is used (see review by Ned 1975). A change in sensitivity to β_2-mediated responses with an unaltered α-receptor sensitivity would then be expected to lead to an altered slope of the dose-response curve in isolated rats with a steeper rising curve. The data of Speiser and Weinstock seem to confirm that such a change in slope occurs. Since the slope of the dose-response curve was unaltered in my trained rats it is not likely that decreased sensitivity to the β_2-action of NA is the mechanism of the observed changes in sensitivity.

We are left, by elimination of the other explanations, with the possibility that quantitative and/or qualitative changes in the α-receptor population or in its link with the contractile response are the most likely mechanisms of the observed increased sensitivity to the vasoconstrictor actions to NA seen in trained rats. What then could be the trigger for such a change in the α-receptor mediated response in vascular smooth muscle? It is known that decentralisation of sympathetic neurons leads to a post-junctional type of supersensitivity to the actions of NA (see review by Trendelenburg, 1972). As the sympathetic nervous activity at rest is lower than normal in at least the heart and the spleen (Section F) and probably also in the vasculature (see Section D) of trained rats, one might speculate that this lowered sympathetic tone might lead to a decentralisation-type of hypersensitivity. However supersensitivity following surgical decentralisation takes more than 48 hours to become measurable and does not reach a maximum until four weeks or more after decentralisation (see Trendelenburg, 1972). Since the trained rats were submitted to daily bouts of exercise throughout the experimental period, there were daily increases in their sympathico-adrenal activity sufficient to lead to excretion over 22 hours of an amount of NA in the urine that was of the same order as (paper III), or larger than (paper II) the amount secreted by the resting control rats. Therefore it would appear that sympathetic nervous activity in chronically exercised rats is not depressed to a sufficient degree for long enough periods for decentralisation supersensitivity to occur.

That the opposite phenomenon can occur, namely that increases in receptor sensitivity can occur in response to increased exposure of the receptor to its agonist appears to have been shown for the

metabolic calorigenic" action of NA both in cold-acclimated rats (Hsieh and Carlson, 1957 for further references see Chapter 4) as well as in rats treated with repeated NA injections (Le Blanc and Pouliot, 1964) and for the isoprenaline-induced increase in O_2—consumptio (Le Blanc *et al* 1972) Such mechanism namely potentiation of the α—receptor response f vascular smooth muscle occurring as result of increased exposure t physiological α—ago ists (NA and adrenaline) would provide a convenient hypothesis to explain the findings the trained group as these rats are subjected t bouts of markedly increased sympathetic nervous activity as well as increased secretory activity f the adrenal medulla, during their daily exercise periods.

There is however one further hypothetical mechanism that deserves consideration. Physical xercise leads to increased secretio f corticosteroids from the adrenal cortex (for ref see Chapter I and Section B) and it is possible that such change i the endocrine status of the animal can alter the structure and/or the umber or function of for xample vascula smooth muscle α—receptors. Changes in effector organ sensitivity to NA following prolonged changes in circulating steroid levels (changes that are present in isolated organs perfused by steroid free medium) have been reported to occur in both directions. Firstly pregnancy (Tesuw and De Jong, 1973 Dogterom and De Jong, 1974) and chronic progesterone treatment (Dogterom and De Jong, 1974) has been showed to lead to reduced vascular sensitivity to the vasoconstr ctor actions of NA. Secondly elevated levels of plasma corticosterone increase the maximum contractile response to NA, and increase both sensitivity and maximum contract le response to acetylcholine in the anococcygeus muscle of rat (Gibson and Pollock, 1975).

The latter authors studied the increase in sensitivity and maximal contractile response of the anococcygeus muscle f the rat occurring after short-term treatment with reserpine ftor morphine withdrawal and after thyroidectomy They found the responses each case to be qualitatively similar and to be prevented by adrenalectomy and concluded that the mechanism behind the increase in sensitivity in ll these xperimental conditions was the increase plasma corticosterone levels known to occur fter these various treatments (see Gibson and Pollock 1975) Carrier nd co-workers have shown that short-term (24—48 hours) treatment with reserpine leads to an increase both sensitivity and in maximal contract le response to NA carotid and femoral artery strips of dogs and to increased pressor responses to NA in rats (see Carrier 1975) They ttributed these changes to decentralization supersensitivity but as they occur more rapidly than true decentralisatio supersensitivity (see Trendelenburg, 1972) he increases in sensitivity and in maximum response may well be associated with th rise plasma corticosterone levels that persists for a prolonged period following reserpine treatment (Gibson and Pollock 1975) It is noteworthy that the qual tative changes in vascular response to NA provoked by short-term reserpine treatment re xactly the same as those seen in chronically xercised rats and that, as discussed Section B, physical exertion leads t ise in plasma corticosterone levels.

The mechanism behind the increase in ma imum contract le response observed the vascular bed of chronically exercised rats is not clear Carrier and co-workers have shown that aortic strips I reserpine pretreated rabbits handle Ca^2 differently from control strips (see Carrier 1975) and Westfall *et al* (1975) have shown that tissue concentrations f ATP rise conjunction with the development of decentralisation supersensitivity in the vas deferens, both after denervation, decentralisation, and after reserpine treatment simila mechanisms could be responsible for th observed increase ma imal contractile strength f vascular smooth muscle in this study

Conclusions. Thus appears lik ly that the increase in vascula sensi ivity t the vasoconstrictor actions of NA seen in chronically xercised rats is compensatory response most likely brought about by events occurring during he acut physiological ress of heavy muscular xertion either by he effects of locally high concentrations of transmi er he receptor sit and/or by the accompanying increase in circulating levels of corticosteroid hormones, in he rat particularly corticosterone.

It is frequently assumed hat increased sensitivi y f an organ always occurs fter chronic low

level of stimulus and that decreased sensitivity may occur after chronically increased stimulus levels (see Fleming, 1976). The vascular bed of the chronically exercised rat appears to form an exception to this rule. In that a regular experimental stimulus which causes the vascular smooth muscle to be exposed to increased release of NA from sympathetic vascular nerves as well as to higher concentrations of circulating NA is nevertheless associated with an *increased* sensitivity of the effector cell. In this experimental condition such change can be regarded as useful adaptation since it means that transmitter stores can be better conserved in situations where there is an extreme demand for a sustained increase in vascular tone because the requirement for NA will be decreased in proportion to the increase in postsynaptic sensitivity.

CONCLUSIONS to Chapter 3

(1) Daily exercise leads to reduction in weight gain which is not seen intermittent (3 times/week) exercise.

(2) Both daily and intermittent exercise leads to the same degree of cardiac hypertrophy provided the exercise load per session is the same. If the training is started early in life before the cardiac cell nuclei have lost their capacity for mitosis the heart of the ex-trained rat remains larger than that of sedentary control even 6 months after cessation of training.

(3) Intermittent exercise causes the same increase in adrenal gland weight as daily exercise does.

(4) Trained rats exhibit decrease in weight of the spleen; this is probably due to reduction lymphoid tissue secondary to the exercise induced elevation of plasma corticosterone level.

(5) The stores of transmitter in noradrenergic neurons in the brain, heart and spleen are increased by daily swim training and cardiac sympathetic neurons in trained rats probably have an increased maximal capacity for NA synthesis as well. It is suggested that this is part of a generalized adaptive response of the neuron to prolonged increases in impulse flow - response which includes increased activity of the enzymes involved in NA synthesis and probably an increase in number of storage vesicles as well. In the heart it is furthermore possible that an actual neoformation of noradrenergic nerve terminals, occurring together with the formation of new capillaries, may contribute to the increase in transmitter content. Whereas some increase in cardiac NA content persists even 6 months after cessation of training, the elevation of NA content in the spleen appears to be transient, disappearing towards the end of 17 week training programme.

The increase in cardiac NA content and concentration is probably less pronounced in rats trained by intermittent (3 times/week) exercise than that seen after daily exercise.

(6) The adrenal medulla also shows an adaptive increase in the catecholamine content of the chromaffin cells following daily exercise and this is more pronounced in the NA containing cells. In intermittently exercised rats the increase in adrenaline content is similar to that seen after daily exercise, whereas the increase in NA content is considerably smaller than that seen in rats trained daily.

(7) The amount of NA excreted in the urine is lower in the rat trained by daily exercise than in sedentary rat, both at rest and during exercise and it is suggested that this reflects a decreased activity in sympathetic nerve terminals in the vasculature. The amount of adrenaline excreted in the urine on the other hand, is the same in trained and sedentary rats during resting conditions. After exercise, however, the increase in urinary adrenaline excretion is much smaller in trained rats. Intermittently exercised rats have urinary excretion of both NA and adrenaline that, after an exercise session, is intermediate between the values seen in trained and sedentary rats. It is argued that this suggests that the reduction in the exercise-induced secretion of adrenaline from the adrenal medulla of trained rats is not simply due to an adaptation to the "emotional" stress of swimming but reflects further adaptation, perhaps in the metabolic responses to adrenaline.

(8) Both central and some peripheral noradrenergic neurons f trained rats show adaptive changes I nervous activity but in opposite directions. Thus the amount of NA turning over the brain is increased I trained rats t rest, whereas the turnover f NA is decreased i the sympathetic neurons of the heart and the spleen During conditions of minimal physical activity there is n fact no detectable turnover of NA the heart d spleen and it must be assumed that this represents complete inhibition of sympathetic tone.

During both continuous and termittent swimming the turnover f NA in the heart is markedly increased compared with th resting values but remains lower in trained rats than in untrained rats. The dopaminergic neurons of the brain do not show an increased DA turnover during exercise, in contrast with the increased turnover seen noradrenergic neurons in th brain, and perhaps because f this there is no alteration f DA turnover the brain f trained rats at rest

(9) The isolated heart from trained rat shows no alteration I intrinsic heart rate, maximal heart rate or sensitivity to the chronotropic and inotropic actions of NA. On the other hand, the vascular bed of trained rats shows adaptive changes consisting of an increased maximal constr ctor response and an ncreased sensitivity to the vasoconstrictor actions f NA, without any indicatio f hypertrophy of the smooth muscle layer in the vascular wall. It is argued that this represents an increase in sensitivity to the α-receptor mediated response brought about by the effects on the vascular smooth muscle either of repeated bursts of exercise-induced NA release or of the exercise-induced elevation f plasma corticosterone levels.

Chapter 4

A COMPARATIVE STUDY OF COLD–ACCLIMATION AND CHRONIC PHYSICAL EXERCISE (paper III)

HISTORICAL BACKGROUND

Since homeothermic organisms must maintain their body temperature at an optimal level the exposure to cold environment means that such an animal must decrease its heat losses by increasing insulation or increase its heat production, or both. Short-term decrease in heat loss can be achieved only by pilo-erection and by superficial vasoconstriction. As already recognised by Claude Bernard (1871) quoted by Leduc (1961) there are two main sources for the increased production of heat in response to cold exposure firstly increased muscular activity and secondly other chemical processes unrelated to muscle work. When an animal is first exposed to a cold environment (in the temperature range of 1 to 6 C) there is marked increase in muscle tone and regular shivering commences in order to augment heat production as the exposure becomes chronic, heat production remains elevated but the shivering declines and after about 4 weeks it disappears altogether (Sellers *et al* 1954 Hart *et al* 1956) Such an animal has acclimated to the cold environment by increasing its capacity for non-shivering thermogenesis and subsequently non-shivering thermogenesis takes precedence over shivering thermogenesis as the preferred method of increased heat production.

Role of the sympatho-adrenal system in cold-acclimation

Early work of Hartmann *et al* (1923) and Cannon *et al* (1927) showed that the circulating levels of catecholamines were increased by cold exposure, and these workers suggested that the adrenal medulla was a major source of these catecholamines however the studies of Cannon and co-workers on surgically sympathectomized cats (see Cannon 1932) also demonstrated that their ability to maintain their body temperature on cold exposure was impaired and thus suggested role for the sympathetic nervous system as well. Subsequently it has been confirmed that the secretory activity of both the sympathetic nervous system and the adrenal medulla is increased by cold exposure. The sympathetic nervous system largely serves as the "first line of the defence" with perhaps supportive role for the adrenal medullary secretion of adrenaline in the maintenance of normal blood glucose levels marked increase in adrenal medullary secretion of adrenaline represents the "second line of defence" when increased activity of the sympathetic nervous system has failed to maintain homeostasis (see reviews by Leduc 1961 Himms–Hagen, 1976) The increased activity of the sympathetic nervous system in cold exposure serves several purposes First, increased sympathetic nervous activity is responsible for improving the insulation by pilo-erection and by superficial vasoconstriction. Secondly it is involved in the necessary cardiovascular readjustments with increased sympathetic nervous activity in the heart (Ohnnero and Stjerne 1966 Gordon *et al* 1966[b] Shagat and Friedman, 1969) and redistribution of the increased cardiac output in general from skin to viscera (Jansky and Hart, 1968) Thirdly it is involved in non-shivering thermogenesis both by actually initiating the process and by mobilizing substrate (see review by Himms–Hagen, 1976)

Changed calorigenic response

The initial observation that cold acclimated rats had an enhanced calorigenic response to adrenaline was made by Ring (1942) and confirmed by Hsieh and Carlson (1957), and Swanson (1957), and

subsequent work also showed a striking enhancement of the calorigenic response to NA in cold-acclimated rats (Hsieh and Carlson, 1957 Depocas, 1960 and many later authors see review by Himms-Hagen 1976) Although the maximal calorigenic response to both NA and adrenaline is increased to an equal degree in cold-acclimated rats, it has been shown that in this species NA is normally the main chemical mediator of non-shivering thermogenesis (see reviews by Leduc, 1961 and Himms-Hagen, 1976) During continued cold-exposure the capacity for non-shivering thermogenesis increases and reaches a ma imum after about 4 weeks so that a cold-acclimated rat can maintain its body temperature in a cold environment even when paralyzed by curare, whereas a warm-acclimated animal under the same conditions rapidly becomes hypothermic (Cottle and Carlson, 1956)

Brown adipose tissue hypertrophies in cold-acclimated rats and is assumed to be site of non-shivering thermogenesis, however the major part of non-shivering thermogenesis is likely to occur in skeletal muscle (see review by Himms-Hagen, 1976)

The calorigenic effect of a standard dose of exogenous NA increases rapidly during the first five days of cold exposure, thereafter it increases more slowly and a plateau is reached for about 30–40 days of cold exposure (Jansky *et al* 1967) other words at the same t me as they cease to shiver The size of the maximum metabolic response given by a standard dose of NA is however inversely proportional to the temperature of accl mation, so that the lower the temperature the higher the final maximal calorigenic response. The time course of the acclimation process is however identical at different temperatures, at least within the range –1 C to +15 C (Jansky *et al* 1967) Le Blanc and co-workers have shown that similar increases in calorigenic response to catecholamines with an accompanying increase in cold-tolerance, can be achieved by daily injections f NA (Le Blanc and Pouliot, 1964) or isoprenaline (Le Blanc *et al* 1972) and it appears, therefore that it is the increased activity of the sympathetic nervous system that actually triggers the development of the adaptive increase in the total capacity for non-shiver ng thermogenesis.

Urinary excretion and tissue levels of catecholamines

The excretion of NA and adrenaline in the urine is increased during acute cold exposure. The elevated urinary excretion of NA persists throughout the duration of cold exposure but as the rats gradually become acclimated, the amount of NA excreted decreases significantly below the level at the onset of cold exposure and finally stabilizes at a value about three times that of controls the excretion of adrenaline eventually declines to a level about 50–100% above that of warm-acclimated rats at room temperature (see review by Leduc, 1961) As the heat production remains equally elevated this would seem to indicate that an increase in the sensitivity to the calorigenic actions of NA and adrenaline, as well as an increase in the maximal capacity to espond, has occurred in cold acclimated rats. Such an increase in sensitivity has also been demonstrated by Jansky *et al* (1969)

During acute cold exposure the adrenaline content of the adrenal medulla is usually decreased, within 1 day but after about 6 days of continued cold exposure the adrenaline content is higher than before exposure and in most studies remains elevated throughout cold xposure (see review by Himms-Hagen, 1976). The NA content of the adrenal gland is decreased only by severe cold exposure (Leduc, 1961) and in cold-acclimated rats the adrenal NA content has been variously reported as normal (Leduc, 1961) or elevated (Des Marais and Dugal 1951 Moore *et al* 1961 and Kvetnansky *et al* 1971). Acute cold exposure also decreases the NA content of heart, liver spleen, muscle and brown adipose tissue (see review by Himms-Hagen 1976) However on the si th day of continued cold exposure, NA concentrations in heart, spleen, liver and muscle tended to be elevated, whereas fter complete cold acclimation the organ concentrations of NA were no longer significantly bove normal (Leduc, 1961) unfortunately the total organ content of NA was not reported in this study

Acute cold-exposure increases the rate of NA turnover in the brain (Simmonds, 1969) but the NA

content of brain and hypothalamus is generally reported unchanged after moderate degree f cold exposure (Moore *et al* 1961; Gordon *et al* 1966[b] Co nd Potkonjak, 1967) nd reported as increased (Ingenito and Bonnycastle 1967) fter prolonged cold xposure.

Organ and body growth in cold-acclimated rats

Some but far from all previous studies have reported decreased weight gain in cold-acclimated rats (Schwabe *et al* 1938 Heroux and Hart, 1954 Heroux and Gridgeman, 1958 Strømme and Hammel, 1967) whereas on the other hand cold-acclimated rats display hypertrophy of heart, adrenal glands, thyroid gland kidney liver and intestinal tract (Emery *et al* 1940; Heroux nd Hart, 1954 Herou and Gridgeman 1958) The decreased body weight is mainly accounted for by reduced skeletal muscle mass, but the weights of total fat tissue skeleton and spleen are also reduced (Heroux and Gridgeman 1958) and, occasionally total body length has been reported to be slightly below control values (Emery *et al* 1940) It should however be pointed out that reduction in skeletal muscle mass does not occur in wild *rattus norwegicus* which accl mates to cold during the winter and the only significant change in organ weight in these rats is cardiac hypertrophy (Heroux, 1961).

Physical fitness and cold-acclimation

That man may acclimate to cold xposure in the same way as small mammals by increasing his metabolic heat production has been claimed by several investigators (Burton, 1949 Adams and Heberling, 1958 Scholander *et al* 1958) However most studies on cold-acclimation in man have been field studies where increased levels f physical activity have been unavoidable and it was suggested by Adams and Heberling (1958) that many of the findings taken to indicate cold-acclimation in man might have been due to change in physical fitness alone In support of th suggestion, the same authors reported that fter three week programme of physical training the trained subjects maintained higher skin temperatures and had levated heat production when compared with control subjects in cold test and these observations were partly confirmed by Keatinge (1961) Later Heberling and Adams (1961) reported that following an extended programme of physical training subsequent period of cold xposure (winter bivouac in Alaska) did not produce any further improvement in cold tolerance and similar conclusions were reached by Lange Andersen (1966)

The inherent d fficulty in doing th type f study humans is the problem of standardizing the cold xposure and achieving strictly comparable control group and these problems are f course much easier solved in laboratory studies on small mammals. One such study dealing with the relationship between physical fitness and cold acclimation demonstrated that chronically xercised rats, like physically fit men xh bited considerably greater heat production and maintained higher skin temperatures on their feet during cold xposure tha control rats did, lthough during severe cold xposure the chronically xercised rats were not able t maintain their skin temperature quite as well as cold-acclimated rats (Strømme and Hammel, 1967)

Plan f experiments

Thus there are many indications that the process f cold-acclimatio has several mechanisms in common with adaptation to chronic exercise and the study paper III was designed to compare directly some aspects of the sympathetic nervous system in these two daptive conditions.

In this study one group of rats was trained by chronic exercise (swimming) and second group was cold-acclimated at 4 C. These xperimental groups were compared with warm-acclimated control rats. In the studies on urinary CA xcretion after acute xercise the chronically exercised rats had been sedentary for 48 h and the cold-acclimated rats had been at room temperature for 48 h.

RESULTS (paper III)

Body and organ weights

There was no change f nal body length in any of th groups, but as mentioned earlier the mean body weight of trained rats was 7% less than that of controls the mean body weight of the cold-acclimated rats was in between these two groups and not significantly different from either group. Both cold-accl mated rats and exercised rats displayed cardiac hypertrophy f the same magnitude, with mean heart weights 7% heavier tha that of the control group. Submandibula glands were significantly hypertrophied in the cold-accl mated group compared with both controls and exercised rats, with a weight increase of 13% compared with the control value. The weight of the spleen I cold-acclimated rats was no different from that of controls, whereas the decreased spleen weight in the trained rats was statistically significant only when compared with the cold-acclimated group (−9%). Both trained and cold-acclimated rats displayed hypertrophy of the adrenal gland (+8% and +13% respectively).

Catecholamine content of the organs

The NA content of the bra was unaltered in the cold-accl mated rats (controls 0.64 ± 0.02 µg NA, n 12 cold-acclimated 0.65 ± 0.04 µg NA, 11 previously unpublished) The total NA content of the heart was sign ficantly increased in both trained (28%) and cold-accl mated (+ 18%) rats whereas cardiac NA concentration was significantly increased only in trained rats (+ 18%). The total NA content in submandibular glands was significantly increased in cold-accl mated rats (+ 19%), whereas the NA concentration in the gland was not significantly altered any group. The total content f NA in the spleen was elevated (+ 28%) in trained rats but decreased (− 14%) in cold-acclimated rats. The total content of adrenaline in the adrenal gland was significantly ncreased only in the trained group (13%) although the adrenal ne content also tended to be somewhat elevated above control values (+ 10%) in the cold-acclimated group. The drenal NA content was markedly increased (+ 32%) in both trained and cold-accl mated rats and consequently the proportion f the total CA content of the adrenal gland that is comprised of NA is significantly elevated both trained nd cold-accl mated rats.

Urinary excretion of catecholamines

During *esting conditions* both trained and cold accl mated rats excreted significantly less NA in th urine − 33% and − 31% respectively than did control rats. There was no difference between the urinary xcretion of adrenaline between trained rats nd controls, but the cold-accl mated group the excretion of adrenaline was 60% lower than that of controls. *After exercise* all groups xcreted signif cantly more NA than during rest, but only controls and cold-accl mated rats excreted significantly more adrenaline However the amounts of NA xcreted after xercise were different in the three groups. Thus the trained rats xcreted 36% less NA after exercise than th control group and 28% less than the cold-acclimated group the cold-accl mated rats in their turn lso excreted significantly less NA (− 15%) than the controls did When paired observations from the same animal were studied it was found that the net increase in the amount of NA xcreted fter xercise (i.e. fter subtracting the amount of NA xcreted during rest period) was of the same magnitude in cold-acclimated rats and controls, but significantly smaller (− 42%) in trained animals During *cold stress* the excretion of both NA and adrenaline in the urine was markedly increased compared with the resting values in ll the experimental groups. The xcretion of adrenal ne was of the same order of magnitude all groups, whereas the amount f NA e creted in he urine by both the cold-acclimated and the trained rat was significantly lower than that f the control group − 17% and − 20% respectively

DISCUSSION

The findings i trained rats are discussed in detail in Chapter 3 and will only be further discussed here in relation to the observations on the cold-acclimated rats.

Body and organ weights

In this study the marked inhibition of weight gai in cold-acclimated animals reported by several previous studies (for references see Introduction) is not seen. This is probably mainly due to the fact that in our experiment the severity of the cold stress was somewhat diminished by keeping the cold-acclimated rats in the same way as the controls and exercised rats, i.e. in cages with bedding, 5 to 6 rats per cage thus allowing some protection. Strain differences could also be another influencing factor since crossbred Sprague—Dawley—Wistar rats are much more resistant t inhibition of growth by cold-acclimation than are Wistar rats (Heroux and Hart 1954) Finally it should be recalled that although the body weight of the cold-acclimated rats did not differ significantly from that of the controls t did not differ significantly from the body weight of th trained rats either This suggests that there moderate decrease in body weight of our cold-acclimated rats.

The cold-acclimated rats were found to have the same degree of cardiac hypertrophy as the trained rats, which is hardly surprising in view f the considerable increase in cardiac output during cold exposure found in both warm- and cold-accl mated rats (Jansky and Hart, 1968) Cold exposure to temperature no more severe than + 10°C gives rise to increases in cardiac output, stroke volume and heart rate of the same degree as does running at speed of 20 m/min in warm-acclimated, untrained rats (Popovic *et al* 1969) and increased activity in the sympathetic nerves to the heart is shown by the finding that acute exposure to cold as well as to exercise increases cardiac NA turnover (for references see Historical Background)

As mentioned in the Historical Background the influence f cold-accl mation on the size f different organs has been extensively studied. However one organ which seems so far to have escaped attention is the salivary gland. In view of the increased food intake f cold accl mated rats (Schwabe *et al* 1938) and the fact that liver nd digestive tract have been found to weigh more in cold-acclimated rats (Heroux and Gridgeman, 1958) the finding that the submandibular glands f cold-accl mated rats significantly increase in weight is not very surprising. It is however noteworthy that the total amount of NA in the submandibular glands f cold-accl mated rats is also significantly increased, thus maintaining an unchanged transmitter concentration. Catecholamines induce the secretion of amylase and the enlargement f salivary glands occurring in response to tooth amputation is mediated by the sympathetic nervous system, furthermore large doses of isoprenaline cause hypertrophy and hyperplasia of salivary glands in rats (see review by Hamma—Hagen 1972). Thus reasonable interpretation of these findings would be that the enlargement f the submandibular glands in cold-acclimated rats is related to trophic influence of increased sympathetic nervous activity secondary to increased food intake and/or to cold-acclimation itself

The increase in weight of the adrenal glands f cold-acclimated rats is in agreement with previous reports (see Historical Background) and this hypertrophy is known to occur within one week f the onset of cold exposure (Herou and Schonbaum, 1959 Kvetnansky *et al* 1971) the weight increase of the gland is due to an increase in the number f cells the zona fasciculata (Heroux and Schonbaum, 1959).

Organ catecholamine content

Possible mechanisms underlying the increase in cardiac NA content in chronically ercised have been discussed in detail previously (see Chapter 3 Section C) It is likely that the same

are involved in the increase of the total NA content of the heart found in cold-acclimated rats, since as mentioned above the activity of cardiac sympathetic nerves is increased by cold xposure and since acute intermittent swimming in + 18 C water (an experimental stress where *cold stress* is the predominant feature) causes increased tyrosine hydroxylase activity in rat stellate ganglia and in the heart (Thoenen *et al* 1973) Thus the increase in NA content i the hearts of cold-acclimated rats also fits the concept of increased sympathetic nervous activity being able to induce the synthesis of increased stores of noradrenergic transmitter as discussed in Chapter 3 Sectio C. The fact that cold-accl mated rats did not show significant increase in the *concentration* f NA in the heart as was found in the trained rats could well be due to the physiological stress being of less severity although constant rather than intermittent in nature then the stress of exercise It need not be a characteristic of cold-acclimation as such.

The finding of decreased NA content in the spleens of cold-acclimated rats is somewhat puzzling perhaps the increased peripheral vasoconstriction combined with high circulating levels of NA, partly of adrenal origin leads to decreased sympathetic nervous activity in the spleen. This suggestion is supported by the data of Jansky and Hart (1968) indicating that the blood flow to the spleen, in contrast to that of most ther vascular beds, is the same in warm- as well as cold-acclimated rats when exposed to 30 C or to 9 C. Thus, the spleen seems not to be actively involved in the circulatory adjust ments following acute or chronic xposure to cold.

The total NA content, and the relative proportion of NA, was significantly increased in the adrenal glands of cold-accl mated rats agreement with the work of Kvetnansky t al (1971) Several authors (see review by H mms—Hagen, 1976) have reported an ncrease in the amount of adrenaline as well as that of NA in the adrenals of cold-acclimated rats, but although we also see tendency towards increased adrenal adrenaline-content the difference is not significant. The increase in the adrenal CA content is l kely to be mediated through induction of enzymes as discussed in Chapter 3 Section C, since the level of adrenal phenylethanolamine N—methyltransferase (PNMT) activity as well as the activities of tyrosine hydroxylase and dopamine β—hydr xylase are increased by cold stress, although both tyrosine hydroxylase and PNMT activity return to control values fter si weeks cold exposure, when cold acclimation has occurred (Kvetnansky *et al* 1971).

The observed increase in total NA content in the submandibula glands of cold-acclimated rats, as already discussed bove is presumably also secondary to increased nervous activity in the sympathetic nerves.

Urinary excretion f catecholamines

Since chronically exercised rats xcreted less NA during period f cold stress than did sedentary warm-acclimated rats, and no more than the cold-acclimated rats xcreted, the possibility arises that chronic exercise can also increase the sensitivity of tissues to the metabol actions f catecholamines as occurs in cold-acclimated rats (see below) This may be due to the daily exposure f the tissues t higher than normal concentrations of catecholamines during exercise periods thus, it is known that daily treatment of rats with NA sensitizes their tissues to the metabolic actions of NA (Le Blanc and Pouliot, 1964) although not t the cardiovascular actions (Le Blanc *et al* 1969) The finding in adipose tissue f chronically xercised rats f an increased NA-sensitive lipolytic activity (Froberg *et al* 1972) is consistent with the above suggestion. However the increased sensitivity to NA in the vascular smooth muscle of chronically exercised rats (paper IV see Section G, Chapter 3) could also contribut to the observed lower increment in NA xcretion in the cold by reducing transmitter demands for the maintenance f blood pressure and for the vascula adjustments that occur during cold exposure (cf Jansky and Hart, 1968)

In a study pertinent to the interpretation of the above findings, Strømme and Hemmer (1967)

report that although chronically exercised rats were able to increase their metabolic rate significantly more than control rats, and just as much as cold-acclimated rats, in response to cold stress and although they maintained higher peripheral skin temperatures during the cold exposure than control rats did, their metabolic response to injected NA nevertheless was the same as that of control rats. They therefore suggested that the enhanced capability of trained rats to elevate the metabolic rate during cold stress was mainly due to an "improved shivering capacity". Unfortunately there are several aspects of their experimental design that make their results difficult to interpret. Firstly they do not state whether the cold-acclimated rats were taken straight from their +5 C environment to the measurements of metabolic rate at various temperatures or whether they were allowed some time at room temperature in between. This is important since it is known that the metabolism of cold-acclimated rats remains elevated for at least 24 h after return to room temperature with a higher than normal urinary excretion of NA (Leduc, 1961) and, as will be discussed below, it probably takes at least 48 h for the metabolic rate of cold-acclimated rats to reach truly basal levels. In the study of Strømme and Hammel (1967) the metabolic rate of cold-acclimated rats at +26°C was almost 60% higher than that of controls suggesting that they had only very recently been removed from the cold environment. This among other things means that the measurements on the cold acclimated group do not start from a true resting level and also means that the blood levels of circulating catecholamines are higher in the cold-acclimated group than in the control group at the time when the various metabolic studies were made. Secondly the oxygen consumption is only measured from the 25th minute after the (subcutaneous) injection of NA and onwards, which means that they might have missed the peak metabolic response which occurs already 15 min. after the same dose given intramuscularly (Hsieh and Carlson 1957). Thirdly and probably less importantly the dose of NA given is calculated per kg BW which means that both the cold-acclimated and the trained rats received smaller doses of NA per animal. As the proportion of total body weight that is made up of "lean body mass" will be different in all three groups, the trained group having smaller fat depots and more skeletal muscle mass, and the cold-acclimated group less skeletal muscle mass, than controls, it is virtually impossible to predict a dose regime which would give equal tissue levels of NA in all three groups. With these reservations in mind it is perhaps not surprising that not only do these authors fail to demonstrate an increased sensitivity to the metabolic effects of injected NA in exercised rats, they also failed to demonstrate the increased sensitivity to the calorigenic effects of NA characteristic of cold-acclimated rats. Admittedly the total metabolic response after the injection of NA was higher in the cold-acclimated group but that was entirely due to the fact that they started off from a 60% higher "resting" level, the net increase in metabolic rate after the injection was of the same magnitude in all the experimental groups. This failure to demonstrate an enhanced sensitivity to the calorigenic effects of NA in the cold-acclimated rats, which clearly were acclimated to cold as shown by other results, suggests that their experimental design was not ideal.

Thus it appears that the question of whether or not chronically exercised rats develop increased sensitivity to the calorigenic effects of NA must be left open for the time being, although there are several indications that this may be the case.

The findings that cold-acclimated rats excrete not only significantly less NA, but also less adrenaline, than control rats do during rest (at room temperature) as well as less NA during cold stress, are seemingly in contrast to the results of Leduc (1961). Leduc found a higher urinary CA-excretion in cold-acclimated rats, when compared with warm-acclimated controls, during the first 24 h after return to room temperature while during the second day the CA-excretion did not differ from that of the controls, however Leduc did not collect urine after the second day. The urine collection in this study was made after the rats had been kept for 48 h at room temperature and thus our results indicate that it takes at least 48 h for cold acclimated rats to reach a truly basal level of sympathetic activity after removal from the cold environment. The depressed levels of urinary CA-excretion in our cold-acclimated rats, when the animals are at room temperature are probably mainly related to the

sensitivity to the calorigenic effects of both NA and adrenaline which has been shown to occur in cold-acclimation (see Historical Background) and which presumably is also largely responsible for the smaller increase in the amount of NA excreted during cold stress n cold-acclimated animals than that found in controls, reported by Leduc (1961) and confirmed in this study

The same net increase in the amount of NA excreted after exercise occurred i cold-accl mated rats as in the controls. The fact that there was a difference between the absolute amounts of NA excreted after exercise is because the cold-acclimated rats start from lower basal level of NA excretion than the co trols do Thus it appears that cold-acclimated rats do not develop any "cross-tolerance" to the circulatory stress of exercise although chronically exercised rats do seem to develop degree of cross-tolerance to the metabolic (and circulatory) stress of cold exposure The reasons fo thi difference are not known. Both groups develop cardiac hypert ophy but confl cting results have been reported concerning the cardiovascular sensitivity to NA in cold-acclimated an mals. Increased blood pressure responses to NA in cold-acclimated rats have been reported by Le Blanc (1960) and Herou (1961) on the contrary Honda *et al* (1962) found a decreased effect on vascula resistance However these studies were ll performed in anaesthetized animals with intact cardiovascula reflexes and probably animals just taken from a cold environment nd thus with elevated blood levels of circulating catecholamines. Hsieh *et al* (1966) the other hand using spinalized rats, found slightly lower sensitivity to vasoconstrictor actions of NA at low dose levels, nd no change at higher dose levels in cold-acclimated rats. Le Blanc *et al* (1972) lso us ng anaesthetized intact rats, reported a sensitization in cold-acclimated rats t the chronotropic, but not to the vascular effects of isoprenaline. Furthermore Harr *et al* (1974) reported that in the isolated atria of cold-exposed rats there is a decreased sensitivity to the chronotropic actions of NA after 7 days cold-exposure after 40—45 days of cold exposure they report that the dose-response curve for the chronotropic actions of NA is shifted to the left, but with a higher maximal response so that the ED_{50}-dose remained unchanged the significance of this observation is not clear however since they seem to lack age-matched control atria for this group. On the other hand Himms—Hagen and Mazurkiewicz—Kwilecki (1970) also using isolated at ia, found no change in the sensitivity to chronotropic and inotropic actions of NA in animals that had been acclimated to cold for 8—9 weeks.

Our observation that cold-acclimated rats responded with the same increment i urinary NA after xercise as did controls is more consistent with the reports suggesting an unchanged sensitivity to cardiovascular actions of NA, but other explanations cannot be xcluded. Thus, another possibil ty is that the cold-acclimated laboratory rat is poorly adapted to muscular performance since these rats have a reduced mass of skeletal muscle (Herou and Gridgeman 1958) and since a characteristic of the circulatory adjustment to cold-acclimation is shift in distribution of blood flow from carcass and skin to internal organs and adipose tissues (Jansky and Hart, 1968) It is theref re conceivable although perhaps somewhat far-fetched that the increased "stress" f having t perform physical xercise with a smaller skeletal muscle mass would off-set the decreased NA secretion from the cardiovascular system resulting from an increased sensitivity to cardiovascular actions f NA, and thus result in a unaltered total NA xcretion fter xercise. However the body weights f our cold-acclimated rats were not significantly below those of control rats and it is therefore by no means certain that these particular cold-acclimated rats did have a reduced muscle mass In summary therefore it would appear most l kely that the sensitivity to cardiovascular actions f NA was not increased in our cold-acclimated rats.

It may appear surprising hat the vascular bed of cold-accl mated rats, exposed to elevated concentrations of NA and corticosterone (see Himms—Hagen, 1976) should not develop an increased sensitivity to NA when that f he chronically trained rat does (see Section G Chapter 3) At least two factors could xplain such possible d fference Firstly cold exposure causes only modest increase in circulating NA levels as reflected by an about 3 to 3 6-fold increase in the urinary xcretion (paper III) which is fairly evenly distributed over 24 hours acut xercise on the other hand, causes peak

increase in circulating NA levels which can range from 6 to 60-fold (see review by von Euler 1974). It seems likely that the serum corticosterone levels might also show a similar pattern if one compares cold-acclimated rats to trained rats. Secondly the source of the NA overflowing into the circulation is probably to a large extent different. In cold-acclimated rat a considerable proportion of the NA is likely to come from increased sympathetic nervous activity in brown and white adipose tissue as well as the sympathetic nerves causing pilo-erection and vaso-constriction in the skin (see Jansky and Hart, 1968, and Himms—Hagen 1975). In the exercising rat, on the other hand, a large proportion of the NA probably arises from increased sympathetic nervous activity in vasomotor nerves through activation of baroreceptor homeostatic mechanisms to prevent hypotension due to muscle vasodilatation (see reviews by Bevegard and Shepherd, 1967 and von Euler 1974). Thus it seems very likely that the major part of the vascular bed of the trained rat will have been exposed to very much higher peak concentrations of NA than that of the cold-acclimated rat.

CONCLUSIONS to Chapter 4

(1) Cold-acclimated rats show the same degree of cardiac and adrenal hypertrophy as trained rats but in addition also show significant hypertrophy of the submandibular salivary glands.

(2) Cold-acclimated rats show an increase in NA content in sympathetic nerve terminals in the heart and the submandibular glands; the NA concentration per g organ weight is however not significantly increased and the increase in cardiac NA content is not as great as that seen in trained rats.

(3) In a room temperature environment the cold-acclimated rat excretes less NA in the urine than a warm-acclimated control and the reduction in NA excretion is of the same magnitude as that seen in trained rats. However, in contrast to trained rats, the cold-acclimated rat also shows a reduction in the excretion of adrenaline in the urine in a room-temperature environment. It is argued that this reduction in urinary excretion of catecholamines in cold-acclimated rats at rest reflects the increased sensitivity to the calorigenic actions of catecholamines that characterizes the cold-acclimated animal.

(4) Following a bout of exercise the cold-acclimated rat excretes less NA in the urine than a warm-acclimated untrained rat, but more than a trained rat. In fact, the actual increment in NA excretion in the urine compared with the resting value is the same as that seen in warm-acclimated untrained rats but larger than that in trained rats. It is, therefore, suggested that cold-acclimated rats have no "cross-tolerance" to the largely circulatory stress of exercise and that they probably do not have the increased sensitivity to vasoconstrictor actions of NA that trained rats possess.

(5) During cold exposure the excretion of both NA and adrenaline in the urine is increased in all three groups, however both cold-acclimated and trained rats show a smaller rise in the urinary excretion of NA than that seen in warm-acclimated untrained rats. It is suggested that trained rats may display an increased sensitivity to the calorigenic actions of NA similar to that seen in cold-acclimated rats and that regular physical exercise might confer some "cross-tolerance" to cold stress.

STUDIES ON THE TURNOVER OF NORADRENALINE IN BRAIN AND HEART BASED ON SYNTHESIS INHIBITION BY α-METHYLTYROSINE

(previously unpublished data)

The fact that in steady state conditions the rate of synthesis of NA equals the turnover rate led Brodie *et al* (1966) to suggest that the blockade of NA synthesis *in vivo* following administration of α-methyltyrosine (Spector *et al* 1965) could provide a convenient method for the study of NA turnover by applying steady state kinetics and using the rate constant for the decline in endogenous NA levels as an estimate of the rate constant for NA turnover. This method for turnover estimation has become widely used because of the following advantages: 1) it is cheap, compared with isotope methods, and easy to use; 2) turnover in brain as well as in peripheral tissues can be studied; 3) both NA and DA turnover can be studied; 4) it avoids the pitfalls of the isotope methods in which there might be an unequal distribution of labelled amine between hypothetical "readily available" and "storage" pools. Accordingly this method has been applied to study the turnover of catecholamines in the brain and in peripheral organs of rats trained by physical exercise.

METHODS

After intravenous injection of α-methyltyrosine the inhibition of NA synthesis is almost instantaneous (Brodie *et al* 1966). α-methyltyrosine itself is however very poorly soluble and for that reason the methyl ester hydrochloride of DL-α-methyltyrosine (H 44/68, A.B. Hässle) has become widely used. The methylester is rapidly converted *in vivo* to α-methyltyrosine by an esterase (see Corrodi and Hanson, 1966) and even after an i.p. injection the degree of inhibition of NA synthesis is more than 90% within 30 min (Persson and Waldeck, 1970). After a single injection of 200 mg/kg BW the levels of α-methyltyrosine in the rat brain remain high, well above inhibitory concentrations, for at least 6 h without any tendency to decline (Brodie *et al* 1966). In my experiments H 44/68 was given in an initial dose of 250 mg/kg BW i.p. (corresponding to 200 mg/kg of α-methyltyrosine) followed by 125 mg/kg i.p. every 6 h in order to secure sufficiently high organ concentrations throughout one experiment. This dose regime has in fact been shown not only to ensure inhibitory tissue concentrations of α-methyltyrosine throughout the experiment, but to lead to a continuous accumulation of α-methyltyrosine in various tissues of the rat (Swedin 1970).

Male Sprague-Dawley rats initially weighing about 180 g were divided in three groups, one of which was subjected to daily exercise in the form of swimming seven days per week, with the daily swimming period stepwise increased from 1 h to 2 h, during 14 weeks. A second group was acclimated to 4 C and the third group served as controls. At the time of the turnover experiments their body weights were about 400–450 g. The turnover study was performed 48 h after the last period of exercise, or removal from the cold environment respectively. H 44/68 was given i.p. dissolved in saline in the doses described above. 12 rats were killed at 0 h (no H 44/68), 5 rats each at 4 h, 8 h, 12 h and 16 h after the injection. Tissues were homogenized and catecholamines were separated from the extracts by chromatography as described in Chapter 2. NA was assayed according to Chang (1964) since assay according to Euler and Lishajko (1961) yields falsely high NA values, due to the fluorescence of α-methyl DA and α-methyl NA, when oxidation is carried out according to the latter method (Maître 1965).

RESULTS

General observations

There was a gradual onset of sedation, which became noticeable about 4 h after the injection and progressed to an almost catatonic" state in which the rats huddled motionless with closed eyes, neither drinking nor eating. They were capable of moving few steps when provoked but would not remove themselves from a strange environment, or show any exploratory behaviour and limb placed in an unnatural position would not be moved for minutes. They also had a pronounced polyuria and appeared clinically dehydrated towards the end of the xperiments (16 h)

Body and organ weights

The trained rats exhibited the usual cardiac hypertrophy and reduction in body weight compared with the controls (see Chapter 3 Section B) Following the administration of H 44/68 there was loss of body weight, due to dehydration, which had declined by 7% at 16 h An organ with normally high blood content such as th spleen displayed 19% decrease in weight at 16 h whereas the weight f the brain remained unaffected throughout the experiment (see Fig. 6).

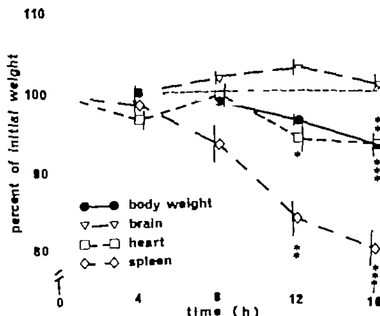


Fig. 6 The effect of α—methyltyrosine-induced dehydration on body and orga weights. Doses f α—methyltyrosine administered as described in the Methods section in this chapter Each value is the mean of 12–14 observations and the vertical bars indicate standard error denotes different from 0 h value $p < 0.01$ different from 0 h value $p < 0.001$

Noradrenaline turnover in brain and heart of trained ats (Fig. 6)

Brain The turnover of NA, as estimated by rate f decline in endogenous NA levels, is f the same magnitude as the seen in the ^{14}C-tyrosine study ($t_{½}$ about 5.5 h) only during the first four hours after synthesis inhibition (later called phase A) with $t_{½}$ f 5.2 h in the control group and f 5.5 h in the

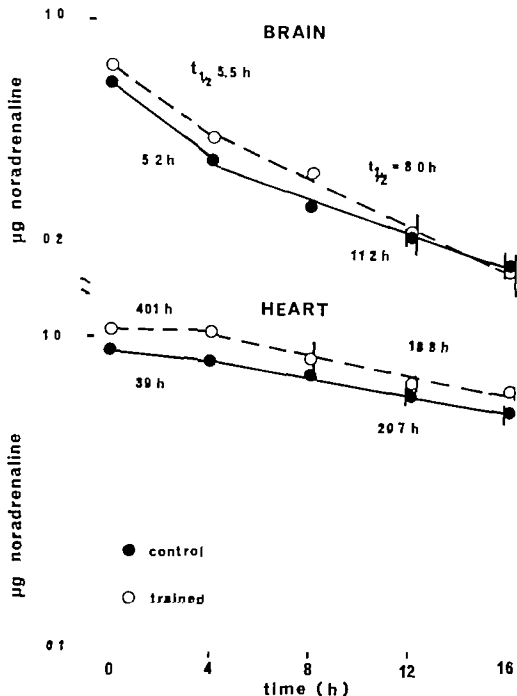


Fig. 6 Disappearence of noradrenaline in brain and heart of chronically exercised rats and of controls following treatment with α–methyltyrosine

The values given are means ± S.E. where vertical bars are absent S.E is smaller than the circumference of the circle. The numbers in the left hand half of the figure are the calculated half-lives (in hours) for th disappearance of noradrenaline during phase A (0–4 h) those in the right hand half are the half-lives during phase B (4–16 h). For dose schedule see Methods section of this chapter

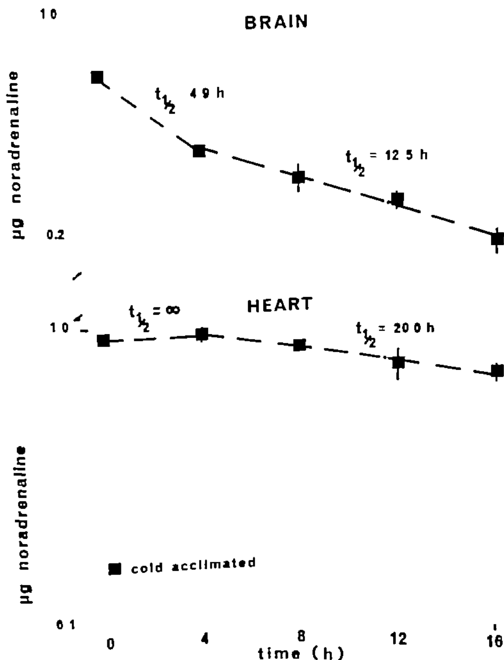


Fig. 7 Disappearance of noradrenaline in the brain and heart of cold-acclimated rats following treatment with α-methyltyrosine.

The values given are mean ± S.E., where vertical bars are absent, the S.E. falls within the area of the square. The half-lives in the left hand half of the figure are the calculated half-lives for the disappearance of noradrenaline (in hours) during phase A (0–4 h) and those in the right hand half are the half-lives during phase B (4–16 h). For dose schedule see Methods section in this chapter.

trained group (n.s.) Subsequently during the 4 h – 16 h time interval (phase B) when the NA content of the brain has been depleted to 60% or less f the original level the fractional turnover rate (slope) is slowed down to give a $t_{½}$ of 11.2 h in controls ($p < 0.001$ v phase A) and to $t_{½}$ of 8.0 h in the trained rats ($p < 0.001$ phase A phase B control v phase B trained: n.s.)

Hearts Again during the first 4 hours the main findings (although not the xact values of the half lives) are the same as with the isotope methods (see Chapter 3 Section F) Thus there is significantly slower turnover of NA in the hearts of chronically xercised rats, in which the estimated $t_{½}$ was 401 h compared with the hearts of controls, where the estimated $t_{½}$ was 39 h ($p < 0.05$ v trained) However during the 4 h – 16 h time interval (phase B) the turnover rate increases significantly i both groups, but more so in trained rats, so that during phase B the cardiac NA turnover is essentially the same in trained and untrained rats with $t_{½}$ f 20.7 h ($p < 0.02$ v phase A) for the control group and $t_{½}$ f 18.8 h ($p < 0.001$ phase A) for the trained group

Cold-acclimated rats. That the above findings were not chance results is shown by the very similar appearance of turnover curves obtained by the same method in rats acclimated to cold as described in Chapter 4 (see Fig. 7) It is noteworthy that cold-acclimated rats, l ke trained rats show a non-significant turnover of noradrenaline i the heart in phase A, with $t_{½}$ of infinity ($p < 0.05$ controls), whereas in phase B the turnover rate accelerates, as in trained rats, to become indistinguishable from that of controls at a half life of 20 h

DISCUSSION

The results f the turnover studies performed by inhibition f NA synthesis are f interest in relation to the discussion in Chapter 7 concerning the possible inverse correlation between the activity f noradrenergic neurons in the lower brain stem and the activity f peripheral sympathetic neurons, notably those nnervating the heart. The regression lines for the decline in endogenous NA concentration versus time are distinctly biphasic in both brain nd heart and it is noteworthy that the slowing of the turnover of NA in the bra occurs at the same t me as th ncrease in cardiac NA turnover rate. It is very tempting to interpret these findings in terms of causal relation In other words: the decrease in sympathetic nervous activity seen for xample in hearts of chronically xercised rats might be dependent on the increased activity of certain central noradrenergic neurons nd when the functions of these neurons are impaired, by loss of transmitter store or secondarily through involvement of other adrenergic pathways, the difference in per pheral sympathetic nervous activity between tra ned and untrained rats disappears. Before further discussion of this idea it must first be established that the biphasic curves are not artefacts due to xperimental condition

Turnover of noradrenaline in the brain

Insufficient synthesis inhibition by α–methyltyrosine cannot xplai the biphasic curve for the decline in NA content firstly because it is during the initial 4 h, when tissue concentrations of α–methyltyrosine might have been suboptimal during the first 30 min, that the apid turnover phase occurs and this is lso the phase where the rate f NA turnover found is in good agreement with the turnover rate found in the ^{14}C-tyrosine study and with turnover rates obtained by other workers (Iversen and Glowinski, 1966 Persson 1969 Taylor and Laverty 1969 Nyback and Sedvall 1970). Secondly in the 4–16 h interval when the turnover rate has slowed down, the tissue levels of α–methyltyrosine are not only supra-optimal they are continuously increasing with the dose regime used in the present xperiment (Swedin 1970) Neither is it likely that this biphasic appearance is due to the

hypothermia that rats treated with α–methyltyrosine develop (Moore *et al* 1967) since hypothermia does not affect whole brain turnover of NA (Corrodi *et al* 1967)

Why then does the decline of endogenous NA follow roughly biphasic regression curve? One theoretical possibility is that the decline of NA levels in the whole brain is in fact the sum of the turnover rates of two or more groups of noradrenergic neurons with widely differing turnover rates, one (or more) very fast and one (or more) quite slow. The sum of these two (or more) exponential processes would then result in a composite bi–(or multi)–phasic regression curve. There are two principal arguments against this possibility. First, one would then also expect to find biphasic (or multiphasic) turnover curves in the brain with the same time course with the various methods involving isotope labelling of NA in the brain. This is however not the case, although some experiments appear to show a very short initial phase with more rapid turnover; however, the turnover then becomes exponential already from about 1–1½ h onwards and remains so (Persson, 1969; Nyback and Sedvall, 1970). Second, the biphasic turnover curve for NA is not likely to be due to the differences in fractional turnover rate between various areas of the brain that are known to exist (Simmonds and Iversen, 1969; Simmonds, 1969; see Nyback, 1971) because the $t_{1/2}$ values of these turnover rates only range from 2.6 to 5.4 with the single exception of the anterior hypothalamus where the $t_{1/2}$ is 8.0 h (Simmonds and Iversen, 1969). These differences are not great enough to give rise to a curve such as that in Fig. 6, since to account for such a curve there would have to be a major pool of neurons, accounting for more than 40% of the total content of NA in the brain, with a half life as long as 9–12 h.

One can think of at least two hypothetical explanations of the biphasic appearance of the regression curve: 1) When depletion of the NA stores within central NA neuron reaches a certain critical level this depletion might by itself depress the spontaneous activity and/or excitability of this neuron. 2) Depletion of NA or DA stores (following inhibition of tyrosine hydroxylase) in one particular aminergic pathway which in terms of the total brain CA store is minor but which has a high turnover rate, might through the consequent transmission failure indirectly lead to a decrease in excitatory nerve impulses reaching many other central noradrenergic pathways. It is noteworthy that transmission failure occurs in peripheral vasoconstrictor nerves which still retain 30% of their NA stores (Sedvall and Thorson, 1967): this level of NA store may actually be below the cut-off value for transmission failure since the latter authors did not study transmission for less severe degrees of depletion. Both of the above mentioned hypothetical explanations are compatible with the time course of decline of NA in the brain after administration of α–methyltyrosine shown in Fig. 6 and it is striking that the slowing of the fractional turnover rate in the brains from both groups occurs when the total NA content has decreased by a similar degree, i.e. to 58% (controls) and 60% (trained), of the steady state levels of NA.

A survey of the literature shows several examples of a similar biphasic decline in brain levels of NA after administration of α–methyltyrosine although none of the authors comments on this fact. Thus the data presented by Brodie *et al* (1966) indicate a rapid decline between 2–4 h and a slower undoubtedly exponential, decline between 4 and 16 h in NA levels of the brain of rats.

Likewise Corrodi and co-workers (1966, 1968) presented results that, when plotted semi-logarithmically show a rapid fall in NA levels between 0 and 4 h and a slower decline between 4 and 8 h both in controls and in rats subjected to immobilization stress. Similarly Westfall (1970) gives data showing a more rapid decline in NA content in the brain between 0–4 h than in the subsequent period between 4–8 h.

When the decline in NA levels in the brain during the 0–4 h time period is closely scrutinized in the work of those authors who give observations at 1 h and/or 2 h, most studies (Spector *et al* 1965; Corrodi and Hanson, 1966 and Westfall, 1970) but not all (Porter *et al* 1966), indicate that the decline of NA levels in the 0–4 h time period is somewhat multiphasic rather than exponential, which is what might be expected if the activities of different populations of noradrenergic neurons decrease

for instance a certain degree of depletion f transmitter stores. However the degree of curvilinearity in the 0—4 h interval is so small that the data given by these various authors are always fitted reasonably well by straight line. For the sake of making a statistical comparison between the relative turnover rate of NA during phase A in different groups, it would thus seem to be justified to use an exponential regression line calculated by least square analys as an approximation f whole brain turnover of NA even in the 0—4 h interval The same argument also pplies to phase A in the decline f NA levels in the heart since various authors have found the rate of decline during the 0—4 h interval to be ex ponential (Swedin 1970) or very close to exponential (Spector *et al* 1966 Westfall 1970)

The biphasic appearance of the curve in all th above studies cannot be explained by insufficient drug concentrations n the bra since Brodie *et al* (1966) found high concentrations of the drug in the brain and no tendency towards decrease, up to 8 h fter a single injection of α—methyltyrosine 200 mg/kg Neither does the biphasic curve stem from the use of racemic as opposed to L—α—methyltyrosine since Porter *et al* (1966) give data that, when converted to conventional semi-logarithmic plot, show biphasic decline in brain levels of NA in mice, both fter administration of racemic or of L—α—methyltyrosine again with rapid phase between 0—4 h and a subsequent slower phase

O the other hand, Nakamura *et al* (1971) found undisputedly linear regression curves f NA content in the hypothalamus in the 0—9 h nterval after administration of H 44/68. This could indicate that there are regional differences between the effect of synthesis inhibition by α—methyltyrosine on the nervous activity of different noradrenergic pathways in the brain. Possibly age differences could also play part in this discrepancy because whereas Nakamura *et al* (1971[a]) use quit young rats most ther workers use more adult animals.

It is noteworthy that there appears to be a correlation between the time t which the change from rapid to slower NA turnover occurs in the brain and the time at which some stress induced changes I the turnover of brain NA are abolished, just as there is in the lterations in cardiac NA turnover f trained rats found in my study Thus the data f Corrodi *et al* (1968) reveal that whereas the turnover of NA in the brains of rats subjected to continuous immobilization stress was increased in the 0—4 h interval it was the same as that of controls during the 4—8 h nterval after H 44/68 admi istration, i.e. during the slower part f the "biphasic" curve (plotted from the results given by Corrodi *et al* 1968).

Turnover f noradrenaline in the heart

Turning next to consider the findings in the heart, there are again several publications with data on the decline of endogenous NA content versus time where the results are much better fitted by a curve that is at least biphasic than by a single xponential regressio line. In contrast to the curves in brains, however the initial phase f NA turnover in the heart is the slow phase and the later phase is the one with more rapid rate of decline Such data are given for the control animals e.g. by Spector *et al* (1966) using guinea pigs and by Westfall (1970) Swedin (1970) and Mueller and Thoenen (1971) using rats Common to all these studies, as well as to the curves in Figs. 6 and 7 is the fact that the acceleration of the turnover rate occurs 4—5 h fter the onset f synthesis inhibition — in other words at the same time as the slowing of NA turnover in the brain occurs. Swedi (1970) speculated that the acceleration of NA depletion occurring after 16 h might be due t the formation of α—methylated amines with a direct releasing effect upon endogenous NA stores. However Spector *et al* (1966) were unable to detect formation of α—methyltyramine in guinea pig brain and heart up t 8 h fter the injection of the α—methyltyrosine and thus this xplanation cannot explain the acceleration f the turnover of NA seen fter 4 h in their experiment. Furthermore Maître (1965) showed that the amount of α—methylNA and α—methylDA, neither of which have direct releasing ffect on endogenous NA stores, formed 16 h fter administration of α—methyltyrosine is much higher in the brain than in the

heart. One might then expect that formation of α–methylated metabolites with direct releasing effects, such as e.g. α–methyltyramine, would also be greater in the brain tha in the heart. However in the brain no acceleration of turnover rate but rather the opposite occurs with increasing time. Thus it appears that at least during the time interval studied, 0–16 h the observed acceleration of the rate of decline of the NA content of the heart from about 4 h onwa ds cannot be accounted for by the releasing action of α–methylated metabolites.

There are however many publications where the regression curves of NA versus time in the hearts of both rats and mice appear to decline exponentially after synthesis inhibition by α–methyltyrosine i.e. with data that well fits a singl regression line in semilogarithmic plot (Porter et al 1966 de Champlain et al 1969 Bralet et al 1971 Nakamura et al 1971 a and b and Lammer and Saller 1974). It is noteworthy that all these authors base their regression curves on the decline in NA concentration (μg/g) in heart rather than on decline in total NA content in the heart. This way f expressing the results introduces quantitatively important artefact since during the experiment progressive dehydration occurs secondary to the polyuria resulting from the kidney toxicity f α–methyltyrosine (Moore et al 1967). With increasing dehydration the weights f most organs including the heart, but excluding the brain decreases (see Fig 5) and consequently falsely high values (in relation to dry weight) will be obtained when the NA levels in an organ are xpressed as μg NA/g. If this progressive dehydration led to an exponential decline in organ weight this experimental artefact would, depending on the half-life not necessarily distort the regression line of NA μg/g versus time but could simply give falsely low value of the slope leading to an erroneously high estimate of half life. However as seen in Fig. 5 the decline in body weight is not exponential but starts slowly in the 4–8 h interval and accelerates after 8 h. The decrease in heart weight parallels that in body weight fairly closely although in the f rst 8 h the change in heart weight lags behind that in body weight (see Fig. 5). If the decline in cardiac NA expressed as μg/organ was exponential, then the superimposed dehydration occurring during the later part of the experiment would artefactually lead to an apparent slowing of the turnover rate when the data are xpressed as μg NA/g wet weight. That this slowing is not observed by the authors who base their estimations of turnover rate on NA concentration in the heart rather than total NA content is in fact an indirect indication that there was in fact an acceleration f the total NA turnover in the later part of their experiment. Most f these authors (de Champlain et al 1969 Brales et al 1971 Nakamura et al 1971 a and b and Lammer and Saller 1974) used dosing schedule of α–methyltyrosine which would lead to more rapid build up to high tissue levels of the drug than the dosing schedule used by Svedin (1970) and myself and thus presumably also to an earlier onset of tubular necrosis with ensuing polyuria and dehydratio Thus it is quite possible that in these experiments the onset f dehydration leading to decreased heart weight coincided in time with the acceleration in cardiac NA turnover that was seen after 4 6 h in my work (Figs. 6 and 7) and in the studies, discussed above, of Spector et al (1966) Westfall (1970) Svedin (1970) and Mueller and Thoenen (1971). As result, the change in heart weigh would have masked the increased turnover of NA and so would have led the group of authors who xpressed their results in terms of NA concentration to underestimat the true rate of urnover O any account, it no justified for this group of workers to ignore such changes in organ weights (in heart –8% at 12 h and –7% at 16 h and in spleen –18% at 12 h and –19% at 16 h). In their studies on ransmitter urnover This objection does not apply t the brain, however since the weight of the brain remains unaltered t least in the 0–16 h time interval.

It is interesting to consider in more detail the xper ment of Mueller and Thoenen (1971) on the effect of isoprenaline on NA turnover in the rat heart. Their data for hearts from controls (expressed in μg NA/heart) indicate clearly biphasic decline in NA levels after inhibition of NA synthesis by α–methyltyrosine The faster decline in cardiac NA levels in isoprenaline treated ats, on the other hand, seems to be ruly exponential. Thus appears that where the rate of turnover of NA in the heart is already high, and the turnover of NA in the brain stem probably lower than normal no further

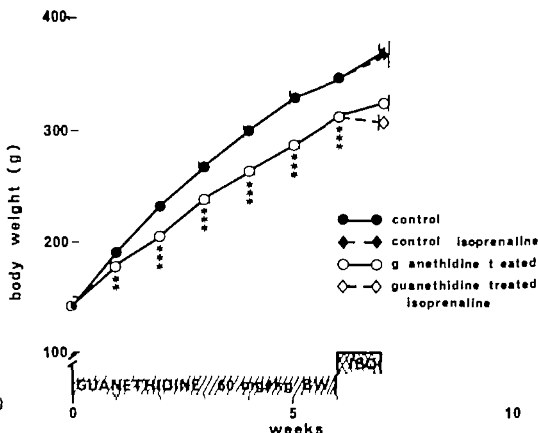


Fig 8. Growth curve of guanethidine and isoprenaline treated mal Wistar rats. Values given are means and vertical bars denote ± S.E. Where vertical bars are absent the S.E. falls within the area of the symbol. The striped bar along the -axis shows the duration of daily treatment with guanethidine (60 mg/kg BW daily i.p.) and the cross-hatched bar shows the duration of treatment with isoprenaline (0.2 mg base/kg BW in arachis oil daily s.c.) denotes different from control, $p < 0.01$ denotes different from control $p < 0.001$

Fig. 9 Stellate ganglia of control and guanethidine-treated (60 mg/kg BW/day i.p. for weeks) rats. 1–2 µm Araldite sections of ganglia removed post mortem and fixed by Immersion in cacodylate-buffered 1% osmium tetroxide pH 7.2. Stain: Methylene blue and Azur II a, b: General view of part of each ganglion X100. c, d. Views at higher magnification (X250) of areas taken from and b. The control ganglion (a, c) has compact structure and is closely packed with neurons and with bundles of nerve fibres with their satellite and Schwann cells. The guanethidine-treated ganglion (b, d) is not smaller than the normal ganglion but has much looser texture: few neurons are seen but almost all of these look normal and are at least as large as the normal neurons. A few are smaller and have displaced nuclei nd poorly defined Nissl material. The intervals between the surviving neurons contain rather irregular bundles f nerve fibres and Schwann cells in an xpanded intraganglionic tissue space which shows lso light infiltration by lymphocytes (small round cells arrows, d) and a few mononuclear cells. At the periphery of the ganglion in one place (b, between arrowheads, upper right) the capsular tissue is rather densely infiltrated by small round cells. Such localized heavier accumulations of lymphocytes were also sporadically distributed in all the sympathetic ganglia from the guanethidine-treated rats. (cf Fig. 2 (Burnstock et al 1971) Previously unpublished results (M.R. Matthews and I. Ostman–Smith)


a




c



Experiments on rats treated with isoprenaline

Effect of isoprenaline-treatment per se (see Table 4). The isoprenaline-treatment given did not significantly alter the body weight or the weight of the submandibular gland in non-sympathectomized rats. In the short time of seven days it did however cause an impressive cardiac hypertrophy with 24% increase in heart wet weight. This was accompanied by a probable slight decrease in the proportion of cardiac weight constituted by dry matter as shown by the dry weight percentage (see Table 4). Somewhat unexpected was the finding that the kidney wet weight was 12% lower the isoprenaline-treated animals ($p < 0.05$); furthermore when the effect of individual variations in body size was diminished by xpressing the data as "kidney ratio (g/100 g BW) the decrease is statistically even more significant ($p < 0.01$).

Effect of guanethidine treatment (50 mg/kg BW) per se (see Figs. 8 and 9, Table 4). I the higher dosage employed in these experiments the effect of the guanethidine-treatment on body growth was more pronounced than in paper VI, already two weeks after starting the drug treatment the body weights of the guanethidine treated rats were 12% lower than those of untreated rats, and the relative difference remained at that level throughout the experiment, with the final body weight of the guanethidine treated controls (group C) 13% lower than that f untreated controls (group A). Body growth as reflected by body length s also significantly decreased at this dose schedule: this can be seen if the data from both guanethidine treated groups are pooled and compared with data from both untreated groups, the pooled mean body length (exclusive of tail) of guanethidine treated rats being 23.9 ± 0.2 cm, 3% less than that of the untreated group which was 27.7 ± 0.2 cm ($p < 0.01$). In this experiment the impairment of growth involved most organs to bout the same degree as it did whole body growth xpressed by body weight and so although the weights f the heart (–15%) the kidney (–12%) and the submandibular glands (–18%) were significantly lower when compared with those of these organs from untreated controls (group A) the heart ratio kidney ratio and submandibular gland ratio (organ weight in g/100 g BW) were not significantly altered. Likewise cardiac dry weight as percentage of wet weight was not significantly altered.

General bservations were the same as quoted above from paper VI including the impression of smaller fat deposits.

There was no detectable tyrosine hydroxylase activity in superior cervical ganglia, which with the sensitivity of the method, means that tyrosine hydroxylase activity was decreased by at least 80%. The profound loss of sympathetic ganglion cells in stellate ganglia of the guanethidine-treated group is shown in Fig. 9, and in superior cervical ganglia there were 1 103 neurons/ganglion compared with 32,546 in control rats (M.R. Matthews and M.E. Florey personal communication).

Effect f guanethidine-treatment on ... ... changes. Whereas non-sympathectomized rats withstood the isoprenaline treatment well with no obvious symptoms and no fatalities, the guanethidine-treated rats subjectively were more affected and some animals would lie prostrate on the floor of the cage although they were able to get up and move when disturbed. An increased salivation was not infrequently noted in guanethidine-treated rats following isoprenaline administration. As mentioned above there were several fatalities due to isoprenaline treatment in the guanethidine-treated groups, the majority occurring within one to two hours after the injection but a few occurring many hours later. Those deaths actually witnessed were undoubtedly sudden deaths, probably due to cardiac arrhythmias, and one such rat was actually successfully resuscitated with xternal cardiac massage.

As clearly demonstrated in Fig. 8, the isoprenaline treatment had marked depressing effect on the rats f weight gain in the guanethidine treated rats, causing them to completely stop gaining weight during the isoprenaline treatment this is in contrast to the findings in the non-sympathectomized

The isoprenaline-induced cardiac hypertrophy was not prevented by the chemical sympathectomy: isoprenaline treatment caused 31% increase the heart weight f guanethidine-treated rats compared with a 24% increase in controls. There was a tendency for decreased kidney weight I isoprenaline treated sympathectomized rats (−11% when compared with the guanethidine only group) but this was not statistically significant.

DISCUSSION

Chemical sympathectomy with guanethidine

E tent of sympathectomy The first question that must be considered is the degree of completeness of the sympathetic denervatio particularly in the cardiovascular system which is the most pertinent for the interpretatio of the results in this study The results of the NA estimations I the various organs indicate that the loss of sympathetic innervatio is xtensive but not complete. O the surface it may seem difficult to reconcile findings f remaining NA levels varying between 10 to 17% with the assessment by Burnstock *et al* (1971) that less than 2% f the sympathetic ganglion cells remain intact after the same treatment schedule using rats of the same strain age and six; however Heath t al (1972) bserved that the few sympathetic ganglion cells that remained were hypertrophied and appeared to give rise to a larger umber of processes than normal Furthermore Blythe *et al* (1976) also using the same treatment schedule in Sprague—Dawley rats, reported that in contrast to the f ndings f Burnstock *et al* (1971) they did see some evidence of regeneration of sympathetic nerve terminals, as indicated both by fluorescence histochemistry and NA assays. Thus they reported that whereas there were no detectable adrenergic nerve terminals in the ventral caudal artery 24 h after cessation of the guanethidine treatment lready after two weeks occasional fluorescent terminals were seen in half of the specimens and after ten weeks ll specimens examined showed an adrenergic vascular innervation, lthough still attenuated compared with controls, I the heart there was n detectable NA one week after cessation of guanethidine treatment, but fter two weeks the NA level had I creased to 9% of normal and fter eight weeks the cardiac NA content was 22% f normal lthough at the same time only rare adrenergic fibers, few of which were intensely fluorescent, were found on fluorescence histochemistry in the atria (Blythe *et al* 1976) It is l kely that the patter of reinnervation fter chemical sympathectomy with guanethidine treatment is the same as that seen fter 6—OHDA treatment, in which case the reinnervation of the atrium occurs earlier and is much more complete than the reinnervation of the ventricles which remains largely restricted to vascular innervation (Jacobowit 1975) The association f only rare fluorescent fibers, as seen on fluorescence histochemistry in atria, with cardiac NA content of 22% of normal in the study of Blythe *et al* (1976) might perhaps be xplained terms of piling up of neurotransmitter in regenerating non-terminal xons similar to t seen after ligation (Dahlstrom, 1965) and after 6—OHDA treatment (Malmfors and Sachs, 1968) sidering the above observations it appears that in the present study the denervation of the hear been complete for minimum duration f half of the xperimental period, nd that innervation that occurred in the heart during the second half was small cardiac NA of the experiment reaching 17% of normal and probably largely confined to rai vasculature and to a small xtent of the atria while the ventricular myocardium is had any significant degree of reinnervation.

Considering the vascular bed, reports are again somewhat conflicting. only occasional fluorescent fibers even at four months fter cessation of treatment, (1976) find a regular but sparse reinnervation after 10 weeks (see bove) From of view Blythe *et al* (1976) reported that pressor responses to carotid occlusion were still present fter chronic guanethidine treatment, although reduced in amp

adrenal medulla was left intact, however and the adrenal medulla may contribute to the pressor responses after both these treatments (Heymans and Neil 1958; Lalanne *et al* 1966) Blythe *et al* (1976) did in fact also demonstrate that there was considerable degree of denervation supersensitivity in the vasopressor response to i.v. NA in their guanethidine-treated rats 10 weeks after cessation of treatment, with a six-fold increase in sensitivity at low doses and two-fold increase in sensitivity at high doses of NA, the altered slope of the dose-response curve suggesting abolition of neuronal re-uptake (see Trendelenburg, 1972)

It seems likely therefore that small degree of vascular reinnervation may occur but that within the time period involved in the present study the reinnervation is far from complete and denervation supersensitivity of vascular smooth muscle persists. However Westfall *et al* (1975) have reported that in the denervated vas deferens the number of neural contacts between individual smooth muscle cells increased, and if the same is true also for vascular smooth muscle small degree of reinnervation may have not inconsiderable functional significance This is in agreement with the findings of N.O Sjöstrand and T Sjöstrand (personal communication) that in guanethidine-treated rats the carotid occlusion reflex persisted even after demedullation of the adrenal medulla.

Influence of guanethidine treatment on body and organ growth. Decreased weight gain has been reported in rats treated with guanethidine (Eränkö and Eränkö, 1971 Johnson *et al* 1976) but these authors do not comment on the size of individual organs and no data seems to have been published previously on the effect of chronic guanethidine treatment on body and organ growth in young adult rats. The effect on weight gain may be roughly dose-dependent since treatment with 30 mg/kg BW leads to 7% lowering of body weight (paper VI) 60 mg/kg 13% lowering of body weight (present study) and 100 mg/kg in newborn rats lowers adult body weight by 20–30% (Johnson *et al* 1976) The effect is not related to the immediate effects of guanethidine as such though since it persists after discontinuation of the treatment (refs. as above) and it is not related to non-specific effects of intraperitoneal injections since it is seen also after subcutaneous administration (Johnson *et al* 1976) It is possible that the age at the start of treatment is an influencing factor since the more pronounced weight depression was reported in neonatally treated rats (Johnson *et al* 1976) the less [illegible] moderate result with rats weighing about 140 g (age 6 weeks) at the onset of treatment (present study) and the smallest effect on rats weighing about 180 g (age 7 weeks) at the onset of treatment (paper VI). Several observations suggest that the impairment of weight gain is related to the degree of sympathectomy Firstly oral treatment with high doses of guanethidine (about 100 mg/kg BW/day for 6 weeks) fails to achieve significant sympathectomy and also fails to impair weight gain (E. [illegible] LW Chubb and A D Smith unpublished observation). Secondly long term treatment of [illegible] with the β–blocking agents practolol or propranolol has been reported to result in decreased growth rate (Vaughan Williams and Raine, 1974 Vaughan Williams *et al* 1975) Immunosympathectomy on the other hand has not been reported to interfere with body growth (see Levi–Montalcini and Angeletti, 1966 Clark 1971) but this may be related to the fact that the degree of sympathectomy [illegible] in the gastro-intestinal tract, including the liver is poor (Iversen *et al* 1966 Thoenen 1972)

Any suggestions as to the possible site of action of the growth inhibiting effect of chemical sympathectomy by guanethidine must be purely speculative It is unlikely that the [illegible] changes in stool consistency not really amounting to diarrhoea, would reflect [illegible] severe enough in itself to impair absorption sufficiently to cause malnutrition. As the [illegible] is not normally innervated by adrenergic nerve terminals also appears unlikely [illegible] nervation would influence absorption via interfering with the normal function of [illegible] (see review by Holzbauer and Sharman, 1972). The effects of NA on various organs [illegible] hydrate and lipid metabolism and on protein synthesis such as liver, pancreas [illegible] adipose tissue are complex (see review by Himms–Hagen 1972) and they [illegible]

apparent xplanation as to why chemical sympathectomy should cause partial inhibition of growth, particularly since the noradrenergic innervation in the liver is presumed to be largely vascular and not in the parenchyma (see review by Holzbauer and Sharman, 1972)

When the effect of chemical sympathectomy on the growth of individual organs is considered a quantitative difference between the results in paper VI and the results in the subsequent study using a higher guanethidine dose becomes apparent. In the first study where the retardation of whole body growth is mild and body length not significantly altered, the weight of the heart remains the same as in control rats of the same age and consequently the heart ratio is increased in guanethidine treated rats. I the second study where the nhibitio of growth was more pronounced nd body length was also significantly lower than in controls the heart size was decreased in proportion to the lowering of whole body weight, in other words the heart ratio remained unaltered. This difference could conceivably be due to strai differences in the reactio to chemical sympathectomy by guanethidine, since Sprague—Dawley rats were used the first study and Wistar rats in the second. However it is also possible that this could be xplained by the xistence of a critical level of impairment of anabolic activity presumably through lack of amino acids and energy sources, so that if the supply of "building material" is only slightly reduced normal growth of vital organs continues at the expense of for nstance adipose tissue whereas if the supply is more markedly depressed all anabolic activity is impaired to a roughly equal degree in the absence of preferential demands on organ function. Such an hypothesis is compatible with the observation that if animals are submitted to starvation or marked undernutrition heart weight and body weight decrease in parallel (Jackson, 1925 Va Liere and Sterth 1936 Beznak, 1954) whereas when obese rats are submitted to food restriction with an adequate protein intak body weight decreases more tha heart weight, resulting in an increased heart ratio (Oscai and Holloszy 1970). If the obese rats in the latter study were exercised they lost body weight to the same xtent but without any loss of heart weight, thus demand on organ function appears to play role in determi ing which tissues hypotrophy (Osca nd Holloszy 1970)

Surgical denervation of salivary glands has been reported to cause a decrease in weight of the gland (Wells *et al* 1961) but in both my studies with chemical sympathectomy by guanethidine treatment the decrease in weight of the submandibular gland was proportional t the decrease in whole body weight and thus this may be a non-specific finding. The same is lso true for the lowering f kidney weight found in the second guanethidine study

Urinary excretion of catecholamines. The finding that the urinary excretion of NA remains about half of that seen in control animals is in agreement with what has previously been found in immunosympathectomized rats (for references see bove) A proportion of the NA xcreted in the urine may derive from degree of reinnervatio f the vasculature but most is l kely to derive f om compensatory increase in adrenal medullary secretory activity This assumptio is based on the following observations: ( ) the adrenal content of NA is increased to significantly higher degree than the content of adrenaline (ii) the blood pressure f rats chemically sympathectomized with 6—OHDA falls drastically after adrenalectomy (with steroid substitution) or even just clamping the adrenal vein (de Champlain and van Ameringen, 1973) (iii) immunosympathectomy increases the rate of catecholamine turnover in the adrenal medulla (Iversen *et al* 1966) (iv) chemical sympathectomy with 6—OHDA increases the tyrosine hydroxylase activity in the adrenal gland (Mueller *et al* 1969) Since the urinary excretion of adrenaline remained unchanged in the guanethidine treated rats, it appears that the increased secretory activity of the adrenal medulla is selective and mainly involves NA containing cells. Such mechanism is compatible with the observations that the NA/adrenaline ratio in adrenal venous blood is different after different experimental stimul of adrenal medullary secretion (see review by Lewis 1975) and that hypothalamic st mulation of different areas can induce preferential secretion f one or other f the catecholamines (Folkow and von Euler 1954)

This compensatory adrenal medullary release of NA, together with the denervation supersensitivity of the vascular smooth muscle to NA, and probably some degree of reinnervation of the vasculature and in addition the intact renin-angiotensin system, provide compensatory mechanisms which keep peripheral resistance and blood pressure normal (see de Champlain *et al.* 1976). Le Blanc *et al.* (1969) report normal blood pressure in guanethidine-treated rats (after treatment leading to denervation supersensitivity) and N.O. Sjöstrand and T. Sjöstrand (personal communication) found that under anaesthesia the blood pressure of guanethidine-treated rats (139 ± 1 S.E., mm Hg) did not differ from that of controls (138 ± 8 mm Hg). Blythe *et al.* (1976) state that the blood pressure of guanethidine-treated rats is lowered but they recorded blood pressure under anaesthesia in the carotid artery which, introducing a one-sided carotid-occlusion, normally elevates the blood pressure by about 25—35 mm Hg (Folkow *et al.* 1970). The blood pressure level they report for guanethidine-treated rats (mean blood pressures ranging between 125—140 mm Hg) is in fact clearly above normal and the blood pressure of the controls, mean blood pressure about 155 mm Hg, is distinctly hypertensive. As they showed in the same study that the pressor response to carotid occlusion was decreased in guanethidine treated animals there is no evidence that the resting blood pressure (i.e. before tying off one carotid artery) in their guanethidine-treated rats was lower than normal.

There was a very constant relationship between the urinary excretion of NA of guanethidine-treated rats and that of untreated rats, both during rest (45% of that of the untreated) and after exercise (46%) which means that the guanethidine-treated rats were able to increase the amount of NA released into the circulation when necessary. It appears therefore that the amount of NA excreted in the urine at rest reflects the optimal amount of circulating NA needed to maintain homeostasis. Thus the degree of reduction in NA excretion seen at rest in the guanethidine-treated rats is probably largely determined by the extent of denervation supersensitivity of vascular smooth muscle. It is noteworthy that chemical sympathectomy by inducing denervation supersensitivity abolishes the normal differential between sedentary and trained rats (with lower urinary excretion of NA in chronically exercised rats- see Section D). This is a further indication that the exercise-induced lower level of NA excretion is linked to the increased sensitivity to NA observed in vascular smooth muscle of trained rats (see Section G Chapter 3).

Effect of exercise on chemical sympathectomy with guanethidine. Chronic physical exercise normally produces an increase in cardiac NA content and possible mechanisms of this increase are discussed elsewhere (see Section C, Chapter 3). It is noteworthy that the residual cardiac NA content after chemical sympathectomy with guanethidine was twice as high in the group that was submitted to daily exercise as in the sedentary group. There are several possible explanations for this observation. Firstly it is possible that each individual surviving cardiac noradrenergic neuron is induced by the exercise to increase its NA stores as occurs in the non-sympathectomized animal. Secondly it could be due to increased stores of NA in the cardiac intramural small fluorescent cells that have been described to occur in rats and are commonly called chromaffin cells although they do not always exhibit a positive chromaffin reaction (Jacobowitz 1967). The second explanation appears unlikely however as the fluorescent substance in these cells has not been shown to be NA and it has been suggested that it is dopamine (Eränkö *et al.* 1966). Furthermore cardiac denervation by autotransplantation which still leaves the cardiac chromaffin cells intact (Jacobowitz 1967) reduces cardiac NA content to undetectable amount (Jacobowitz *et al.* 1967). Thirdly it is possible that the exercise-induced increase in cardiac sympathetic nervous activity leads to a decreased susceptibility of the noradrenergic neuron to the toxic effects of the guanethidine treatment as it has been observed that neurons with a low degree of sympathetic ones such as the short noradrenergic neurons of the vas deferens are chemically denervated at a dose level (5 mg/kg BW/day i.p.) which does not produce a denervation of the rest of the sympathetic nervous system (Gannon *et al.* 1971) and, furthermore, decentralization of the superior

cervical ganglion results in increased sensitivity to guanethidine-induced CA depletion (Heath *et al.*, 1972). However, in the spleen, another organ that takes part in the vascular readjustment during exercise, the NA depletion after guanethidine treatment is if anything more pronounced in exercised animals. Fourthly, as the noradrenergic terminals to the vasculature are the first to regenerate (see above) the increased NA content of the heart may be related to increased vascularization which usually follows chronic exercise (reference see Section C); it has not been established, however, whether vascular neoformation in response to chronic exercise still occurs in the absence of adaptive cardiac hypertrophy.

Effect of isoprenaline treatment

I the dose schedule employed i the present study the effect f isoprenaline treatment on the non-sympathectomized animal was restricted to considerable degree of cardiac hypertrophy (+24%) and significant decrease in kidney weight (−12%). Generalized cardiac hypertrophy involving both sides of the heart (see Alpert, 1971) following isoprenaline treatment was first reported by Stanton *et al.* (1969) and this finding has subsequently been confirmed by several authors (Le Blanc *et al.* 1972 and others, see Cohen 1974 for references). Very large doses f isoprenaline (80 mg/kg BW) cause severe gross cardiac necrosis (Rona *et al.* 1959, Stanton *et al.* 1969) presumed to be due to a simultaneous marked increase in metabolic demands and the low perfusio pressure (Stanton *et al.* 1969). More moderate doses, about 5 mg/kg BW, cause not macroscopical infarcts but microscopic lesions with histiocytosis, myocardial cell oedema as well as truly hypertrophied myocardial cells with neoformations of myofilaments are seen (Stanton *et al.* 1969, Pfitzer *et al.*, 1972) and the end result is cardiac hypertrophy with an element of fibrosis (Stanton *et al.* 1969, Bartosova *et al.* 1969). However, low doses of isoprenaline 0.1 mg/kg BW daily – 0.3 mg/kg BW twice daily also produce cardiac hypertrophy (Stanton *et al.* 1969, Le Blanc *et al.* 1972, Cohen 1974) without causing any cellular damage detectabl by light and electron microscopy (Cohen 1974). The significance of the ability of isoprenaline to induce cardiac hypertrophy will be discussed in greater detail below.

The etiology of the observed decrease in kidney weight is obscure, unless the rapid hypertrophy of the heart actually provokes catabolism of other tissues which would be somewhat surprising as the non-sympathectomized rats continued to grow normally during th isoprenaline treatment.

Large systemic doses of isoprenaline 20–50 mg/kg BW twice daily (doses which carry considerable mortality) cause marked hypertrophy and hyperplasia of submandibular and parotid salivary glands, but not of sublingual and lacrimal glands (see review by Himms–Hagen 1972). This effect is blocked by β–blocking agents (Campos and Parr 1968) nd is not prevented by pretreatment with reserpine (Pohto, 1968) or surgical sympathectomy (Campos and Parr 1968), thus it is presumed to be due to a direct β–agonist ffect on the salivary gland. It is noteworthy that isoprenaline as well as NA stimulates secretion f amylase from the salivary gland (see review by H mms–Hagen, 1972). I this study salivary gland hypertrophy did not occur in non-sympathectomized rats fter short term treatment of isoprenaline in dose f 0.2 mg/kg BW dose which nevertheless has marked cardiovascular effects and results in considerable degree of cardiac hypertrophy. I the guanethidine-treated rats, however, there was significant increase in submandibular gland weight following isoprenaline treatment. This confirms the observations of Campos and Parr (1968) that isoprenaline-induced salivary gland hypertrophy is not inhibited by denervation and furthermore it raises the possibility that a denervation supersensitivity may occur for the trophic responses of the salivary gland as well as for secretomotor responses.

Role of the sympathetic nervous system in xercise-induced cardiac hypertrophy

There are two possible explanations for the observation (paper VI) that chemical sympathectomy

by guanethidine-treatment completely abolishes the compensatory cardiac hypertrophy induced by physical training.

(ii) That an increase in work-load is in itself sufficient to evoke cardiac hypertrophy but that the hearts of the guanethidine-treated exercised rats were not subjected to any significant increase in work load.

(iii) The cardiac sympathetic nerves normally release substance which is necessary for the development of exercise-induced adaptive cardiac hypertrophy.

There are several arguments against the first explanation. Not only were the guanethidine-treated rats subjected to the same swimming schedule as the untreated-exercised group, they also displayed the same spontaneous swimming activity. Furthermore, the effectiveness of the exercise is revealed by the fact that the mean body weight of each exercised group was reduced by exactly the same proportion, when compared with that of the respective sedentary control group.

It could be argued that chemical sympathectomy might cause a profound drop in peripheral vascular resistance leading to a decreased work load for the heart. However, several compensatory mechanisms including increased adrenal medullary secretory activity and an activation of the renin-angiotensin and aldosterone systems combine to restore the blood pressure (see Introduction), and although some but not all workers who have measured the blood pressure of immunosympathectomized rats under anaesthesia find slightly subnormal blood pressure (possibly due to impaired ability of the immunosympathectomized rats to compensate for the hypotensive action of anaesthesia), in studies performed on conscious immunosympathectomized rats their blood pressure was found to be normal (see Introduction). If one takes into consideration the degree of denervation supersensitivity to NA in the vascular bed of sympathectomized rats, which is of the order of a two-to-six-fold increase in sensitivity in guanethidine-treated rats (Blythe *et al.* 1976), then the level of circulating NA in the guanethidine-treated rats (as revealed by urinary excretion of NA that is only 45—46% lower than that of controls) is likely to be sufficient to maintain normal peripheral vascular resistance both at rest and during exercise. This is in agreement with the finding of Blythe *et al.* (1976) that rats subjected to chemical sympathectomy with guanethidine treatment were not hypotensive and with the unpublished observations by N.O. Sjostrand and T. Sjostrand (see above) that the blood pressures of such rats are no different from those of control rats when measured by intra-arterial recording under anaesthesia.

Furthermore, if chronic guanethidine treatment did lead to a significant permanent drop in peripheral resistance leading to a decreased work-load for the heart, one would expect, on the work-load hypothesis, that the guanethidine-sedentary rats would have significantly smaller hearts than the untreated-sedentary group; however, the heart weights in these two groups were found to be the same in paper VI.

It could also be argued that the denervated hearts would be unable to increase their cardiac output during exercise sufficiently to cause a significant increase in work-load. However, experiments with dogs do not support this argument. Thus, it has been shown that dogs with chronic cardiac denervation increase their cardiac output on exercise to the same extent as do control dogs (Donald and Shepherd 1964) and studies in racing greyhounds have shown that the capacity for maximal exercise is little reduced by cardiac denervation (Donald, Milburn and Shepherd, 1964). In greyhounds, the increase in cardiac output during the race could be blocked by propranolol in the dogs with cardiac denervation, and these dogs were unable to complete their race (Donald, Ferguson and Milburn, 1968); this indicates that the normal increase in cardiac output observed in dogs with cardiac denervation was achieved through circulating catecholamines, probably largely of adrenal medullary origin.

Thus, we are left with the possibility that the cardiac sympathetic nerves release a substance(s) which is necessary for the induction of adaptive hypertrophy of the heart, or at least the hypertrophy evoked by chronic physical exercise and which might in fact in itself be the factor that induces the

biochemical events leading to hypertrophy f the cardiac muscle cell.

Since sympathetic nerves are known to release not only NA, but also secretory proteins, dopamine β-hydroxylase and chromogranins, as well (Smith *et al* 1970 reviewed by Smith 1973) one cannot from this experiment alone form the hypothesis that NA is the essential link in inducing adaptive cardiac hypertrophy in trained rats. Thus there are three possib lities: either NA itself is sufficient, or the secretory proteins alone are required or both NA and proteins are necessary for the induction of hypertrophy The finding that chronic treatment of rats with low doses of isoprenaline gives rise to cardiac hypertrophy (references see above) supports the general hypothesis and is not inconsistent with the idea that secretory proteins, as well as NA tself could be a factor inducing cardiac hypertrophy since the doses of isoprenaline used cause profound and prolonged drop in blood pressure which would result in reflex activatio of the sympathetic nervous system and increased ctivity in cardiac sympathetic nerves (Stanton *et al* 1969 Mueller and Axelrod, 1968 Mueller and Thoenen, 1971).

The finding that long term treatment f rabbits with either propranolol or practolol reduces the growth of the heart (Vaughan Williams and Raine 1974 Vaughan Williams *et al* 1975) seems to indicate that the β-agonist actio of NA is required for the growth of the heart i the sedentary animal but it should be remembered that propranolol administration decreases the amount of NA released in the heart on stimulation of the stellate ganglion (Naylor and Carson 1973) and thus probably also decreases the amount of secretory proteins released as well as blocking the β-receptors on the heart. Furthermore chronic propranolol treatment probably decreases preganglionic sympathetic nervous activity as well (Raine and Chubb 1977)

The present f nding that the urinary excretion f NA is fairly well maintained in the guanethidine-treated rats and is increased during xercise, probably as a result f increased secretio f NA from the adrenal medulla indicates that the circulating levels of NA are not markedly different in sympathectomized and untreated rats. Thus the reaso why cardiac hypertrophy is prevented by sympathectomy might be because the cardiac muscle cell only hypertrophy ither if they are exposed t the high local concentration f NA reached after release from the nerve endings, if they are exposed to high local concentrations of the secretory proteins.

The fact that chemical sympathectomy did not abolish the cardiac hypertrophy caused by isoprenaline treatment indicates strongly that the trophic factor released from the sympathetic nerves is NA alone. After chemical sympathectomy high local synaptic concentrations of the secretory proteins would not occur: this is because most of the serum dopamine β-hydroxylase activity found i normal rat serum is derived by release from sympathetic nerves, nd little from the drenals (Axelrod, 1972) Therefore by exclusion of the secretory proteins and on the evidence of the ability of isoprenaline to nduce cardiac hypertrophy in the absence of cardiac sympathetic nerves we can conclude that NA must be the normal trophic factor *Although α-agonists have been shown to exert effects on e.g.* myocardial contractility (for references see Williams and Lefkowitz 1978) it seems unlikely that isoprenaline would xert its trophic effect via α-receptors as the ratio of α-agonist activity to β-agonist activity is only 0.001–0.003 1 f this drug (Furchgott, 1972) Furthermore converting the α-agonist activity f the isoprenaline doses given in the present study to an equivalent dose of NA one gets a NA dose in the region f 0 12–0 70 μg per 24 h which is f ly insignificant mount compared with the endogenous concentrations f NA in the circulation, not to mention the very much higher concentrations reached between the sympathetic nerve terminal and the effector cell. If therefore isoprenaline exerts its trophic action via β-receptors it appears highly likely that the trophic action of NA is also mediated via β-receptors

It is noteworthy that the degree of cardiac hypertrophy obtained by isoprenaline treatment was *actually greater in the sympathectomized* rats (31% as compared to 24% in *controls) and* the observation, l ke the findings on salivary glands (see below) raises the possibility that denervation supersensitivity to the trophic actions f NA might occur

Trophic actions of sympathetic nerves and/or catecholamines have been described i salivary glands, where the hypertrophy following repeated tooth amputation has been found to be mediated via the sympathetic nerves (Wells *et al* 1961) and where various catecholamines induce hypertrophy and hyperplasia of the glands (see review by Hamms–Hagen 1972) and in chick embryo where NA has been shown to increase RNA synthesis (Caldarera *et al* 1970) Furthermore it has been suggested that α–blockade with phenoxybenzamine interferes with early stages of liver regeneration in partially hepatectomized rats (Thrower *et al.*, 1973) and that catecholamines affect cell growth and cell division of *Tetrahymena piriformis* possibly via β–receptors (Iwata *et al.*, 1969) It is therefore quite plausible that NA could exert trophic effects on the cardiac muscle cell and in this context the not uncommon occurrence of cardiac hypertrophy in patients suffering from phaeochromocytoma deserves mentioning (Wavell and Crago 1968 Gerola and Jennings, 1972)

In summary therefore it appears likely that in xercise-induced cardiac hypertrophy it is NA released from cardiac sympathetic nerve endings and acting via β–receptors that is the li k between the raised physiological demands and the biochemical events in the cardiac muscle cell leading to compensatory hypertrophy and that increased cardiac work load in itself does not lead to adaptive hypertrophy

CONCLUSIONS to Chapter 6

(1) Chemical sympathectomy produced by chronic guanethidine-treatment is associated with reduction in weight gain and with the higher dose regime reduction actual body growth. There is also reduction in the urinary excretion f NA, the extent f which is likely to be determined by the denervatio supersensitivity f the vasculature Circulating NA is probably largely derived from compensatory selective increase in the secretion f NA from the adrenal medulla. The weight f the adrenal glands in guanethidine-treated rats was increased perhaps indicating that there is increased adrenocortical activity as well.

(2) The chemically sympathectomized rats easily tolerated the exercise programme and the regular physical activity further accentuated the adaptive increase in adrenal NA content seen in sedentary sympathectomized rats. However although the training programme was identical to that in untreated rats, the sympathectomized rats did not show the compensatory cardiac hypertrophy found in the untreated rats. It is argued that this indicates that the adaptive cardiac hypertrophy produced by chronic exercise is not caused by direct effect of the increased work load on the cardiac cell but is instead mediated by release f trophic factor from cardiac sympathetic nerves.

(3) The finding that the generalized cardiac hypertrophy caused by isoprenaline treatment was not bolished by chemical sympathectomy suggests that the trophic factor released from cardiac sympathetic nerves in chronic exercise is NA, rather than any of the secretory vesicle proteins, and that NA xerts its trophic effect on the cardiac muscle cell via β–receptor

Chapter 7

SOME WIDER IMPLICATIONS OF THE RESULTS OF THESE STUDIES

The role of the sympathetic nervous system in the bradycardia of the athlete's heart

It has been a commonly held belief that the relative bradycardia found in athletes and in chronically exercised animals is due to an excessive vagal tone although the experimental evidence for this is both indirect and unconvincing (see Chapter 1). The finding, confirmed by the use of three different methods (Chapters 3 and 5) that the turnover of NA in cardiac sympathetic nerves is significantly lower in trained rats than in controls, both during rest and during exercise, provides an alternative explanation of the bradycardia of the athlete's heart. Since the sensitivity of the isolated heart to the chronotropic actions of NA is not altered in trained rats (Section G, Chapter 3) it would appear that the bradycardia of the trained rat could be accounted for by this decrease in nervous activity of cardiac sympathetic nerves. That a similar situation is found in man is suggested by the observation of Bjurstedt *et al* (1974) that in well-trained human individuals the resting heart rate is not slowed by β-blockade with propranolol.

Further independent evidence supporting the concept of decreased nervous activity in cardiac sympathetic nerves in trained rats is provided by the work of Lin and Horvath (1972). These authors concluded from their experiments in rats treated with propranolol and/or atropine that there is a decrease in both sympathetic and parasympathetic tone to the heart of trained animals, but that the decrease in sympathetic tone is the more prominent.

Ekblom *et al* (1973) found that chemical sympathectomy by 6–OHDA diminished the difference in heart rate during exercise normally seen between trained rats and controls, which supports the concept of lower cardiac sympathetic tone in trained rats. As the difference in heart rate during exercise was not entirely abolished by 6–OHDA treatment the authors concluded that some other factor must be contributing to the observed relative bradycardia. However, since their 6–OHDA treated rats showed evidence of cardiac denervation supersensitivity to circulating catecholamines, the remaining difference in rate after sympathectomy could be entirely explained by the fact that chronically exercised rats, whether trained by swimming (see Section D) or forced running (N.O. Sjostrand *et al* unpublished observation) have a smaller rise in circulating catecholamine levels during exercise than untrained rats do.

As shown in Section G, the intrinsic rate of intact isolated hearts from chronically exercised rats is the same as that of controls, and therefore a decreased intrinsic heart rate is very unlikely to account for the relative bradycardia of the trained rat.

A finding of decreased sympathetic tone to the heart does not, of course, exclude the possibility that a simultaneous increase in vagal tone could be present. However, as atropine causes a smaller rise in heart rate in trained rats than in controls even if the rats have been given propranolol as well (Tipton and Taylor 1965, Lin and Horvath 1972) there seems to be little experimental support for such a concept. Likewise human athletes show a smaller heart rate increase after atropine than controls do (Herxheimer 1921, Kaul 1926).

In summary, therefore, it appears that the bradycardia seen in trained rats is entirely accounted for by the decrease in cardiac sympathetic nervous activity and it seems likely that the same is true in human athletes as well.

The role of training in the treatment of "cardiac neurosis"

It is known that physical training has a beneficial effect on the clinical condition known under various names such as cardiac neurosis" "vaso-regulatory asthenia "hyperkinetic circulation etc with the common denominator f tachycardia, with excessive cardiac output compared to xygen consumption, and frequent benign extra-systoles (Holmgren *et al* 1957[a] 1957[b] Carlsson *et al* 1962 Gorlin 1962) My fi ding that, in rats, chronic physical exercise leads to reduced sympathetic tone to the heart, together with arguments in the preceding section that the same may be true in man, provide reasonable explanation for the clinical mprovement produced by training in this condition. Furthermore, it is lso not unl kely that the effect of training in increasing the vascula sensitivity t NA (see Chapter 3) may play part in improving the "vaso-regulatory asthenia in these patients.

The relationship between the activity f central and peripheral noradrenergic neuron

I Chapters 3 and 5 it was shown that chronically exercised rats exhibit reciprocal changes between the activity in central and many peripheral neurons, thus there is an increase in total transmitter turnover in the brain presumably reflecting increased nervous activity and decrease in NA turnover I many peripheral organs, notably in heart, spleen and probably vasculature. This finding is in accordance with several other reports suggesting an inverse relationship between nervous activity in some central and peripheral noradrenergic neurons. Thus Nakamura *et al* (1971) found that in DOCA—salt hyper tensive rats the turnover f NA is decreased in the hypothalamus and medulla oblongata, unchanged in th rest f the brain but increased in the heart. Conversely the hypotensive action of clonidine and other drugs with α—agonist action has been claimed to be due to the stimulation of central α—receptors leading to decrease in peripheral sympathetic activity (Schmitt *et al* 1968 1971 Anden *et al* 1970 Hoyer and Van Zwieten 1972) The hypotensive action of L—dopa, likely to be due to formation of NA, has been localised to the lower brain stem (Henning *et al* 1972 Schmitt *et al* 1972) Further more Yamori *et al* (1972) found that fter various pharmacological manipulations there was highly significant inverse correlation between brai stem NA content and blood pressure level.

There are at least two possible anatomical sites for an interaction between noradrenergic neurons in the lower brain stem and peripheral sympathetic nervous activity Firstly nucleus tractus solitarii which receives the input from fferent nerves from baro- and chemoreceptors (see review by Calaresu *et al* 1975) has very dense innervation f noradrenergic nerve terminals (Dahlstrom and Fuxe 1965 Bolme *et al* 1972) Secondly microiontophoretic application f NA inhibits sympathetic spinal neurons (De Groat and Ryall, 1967) and there is descending bulbospinal noradrenergic pathway which terminates on preganglionic sympathetic neurons in the sympathetic lateral column (Dahlstrom and F xe 1965)

Thus he reciprocal alterations in nervous activity in central and peripheral noradrenergic neurons f chronically exercised rats fit well with the concept that some central noradrenergic pathway(s) probably the lower brain stem, xert an inhibitory influence on peripheral sympathetic activity That the observed changes in chronically xercised rats are not just coincidental but that the lowering f cardiac sympathetic one seen in fact dependent upon central inhibitory influence by noradrenergic neurons, is suggested by the finding that this lowering of cardiac sympathetic nervous activity disappears when the turnover of NA in the brain slows down as result of synthesis inhibition by α-methyltyrosine (see Chaper 5).

at normotensive levels, and in this respect being if anything more effective than α-methyldopa, it failed to prevent the occurrence of cardiac hypertrophy both in rats treated when hypertension was established and in rats started on treatment prophylactically before hypertension had occurred. The authors interpret their findings as suggesting that the renin-angiotensin system might play a permissive role in enhancing cardiac hypertrophy (Sen *et al.*, 1974). Obviously renin doesn t qualify as candidate for "a final common pathway" in inducing cardiac hypertrophy as compensatory cardiac hypertrophy occurs in "low renin types of hypertensio (see Sen *et al* 1974 fo rats.) However the results of Sen *et al* (1974) f t well with the hypothesis under discussion. Thus α-methyldopa has a central hypotensive action leading to a relative bradycardia as well as a decrease in lumbar sympathetic impulse outflow (Ingenito *et al* 1970 Baum *et al* 1972) It would therefore tend to diminish the xaggerated neurally mediated cardiovascular responses precipitated by external stimuli in spontaneously hyper tensive rats. The hypotensive action f hydralazine on the other hand is predominantly due to relaxation of vascular smooth muscle and both heart rate and stroke volume are increased, presumably due to refle -induced increases in cardiac sympathetic nervous activity as hydralazine-induced tachycardia can be prevented by ganglion blockers and β-adrenergic blocking agents (see Nickerson and Ruedy 1975). Hydralazine would not, therefore diminish pathologically increased activity in cardiac sympathetic nerves in spontaneously hypertensive rats and so according to the hypothesis, would not inhibit the induction of cardiac hypertrophy Similarly Mason *et al* (1968) noted dissociation between the blood pressure control by hydralazine in experimental renovascular hypertension and the persistence of cardiac hypertrophy and this might again be interpreted as supportive evidence for a role of cardiac sympathetic nerves in inducing compensatory cardiac hypertrophy

A further piece f evidence supporting the hypothesis is the finding f Fernandes *et al* (1976) that propranolol treatment reduced the development of cardiac hypertrophy in renal hypertensive rats even though their blood pressure remained as elevated as in the ntreated renal hypertensive rats.

I recent paper pertinent to the hypothesis under discussion Cohen (1974) has suggested that n fact myocardial adrenergic nerve terminals are not required for the cardiac hypertrophy induced by desoxycorticosterone-induced hypertension on the basis of experiments with rats treated with 6-OHDA. However there are several aspects of Cohen s xperiments that can be criticised. First, the dose of 6-OHDA used (20 mg/kg, twice daily on two successive days weekly i.p.) is not sufficient to produce maximal chemical sympathectomy (see Thoenen 1972) Second no allowance was made for the likely regeneration of sympathetic fibres which occurs rapidly in adult animals. Even after much larger doses of 6-OHDA than those used by Cohen (1974) egenerating terminals are seen in the myocardium for 3-6 days (Lorez t al 1975) and after relatively small doses the regeneration is even more rapid (Malmfors and Sachs, 1968 Goldman and Jacobowitz, 1971) Third, the degree of sympathectomy was nly assessed qualitatively by fluorescence histochemistry and th results presented show considerable residual catecholamine-fluorescence in the heart of treated animal Thus, Cohen s (1974) results are not sufficiently critical test of the hypothesis that the cardiac sympathetic nerve is the final common path in many etiologies of cardiac hypertrophy

It can therefore be concluded that there is considerable body of evidence that cardiac sympathetic nervous activity is increased in all the pathological conditions causing adaptive cardiac hyper trophy discussed above and that there are some further indications that this increased nervous activity may initiate the adaptive hypertrophy

What is then the role of ther hormones in cardiac hypertrophy? Patients suffering from pituitary tumours producing growth hormone are frequently noted to develop cardiac hypertrophy (see review by Cohen 1974) and hypophysectomy causes a relative reduction f cardiac size which is only fully reversed if substitution therapy f both growth hormone and thyro ine is given (see review by Wh tehorn 1971). Neither the presence of growth hormone nor that of thyroxine is however necessary

for compensatory hypertrophy of the heart to occur as chronic exercise still induces cardiac hypertrophy in hypophysectomized rats (Tipton and Tcheng, 1971). The situation in skeletal muscle has been studied more extensively and may be analogous. Thus developmental growth of skeletal muscle is dependent on growth hormone and is not prevented by denervation, whereas compensatory growth is dependent on innervation and not prevented by hypophysectomy (see review by Goldberg, 1971). Like growth hormone the presence of thyroxine is necessary for normal postnatal growth and development and the direct growth promoting properties of thyroxine have been well established also in *in vitro* cell cultures of human HeLa cells, human kidney cells and, particularly pertinent to this discussion, in cultures of human myocardial cells (see review by Greenberg *et al.* 1974). It is therefore hardly surprising that xperimentally induced hyperthyroidism causes a generalized cardiac hypertrophy (see review by Korecky and Beznak, 1971). Although hyperthyroidism leads to electrophysiological changes i the myocardial muscle cell that induces higher spontaneous rate in isolated atria (Freedberg *et al.* 1970) the hyperkinetic circulation in hyperthyroidism can be largely normalized by drenergic β—blocking agents (see review by Korecky and Beznak, 1971 Freedberg and Hamolsky 1974 Turner 1974). There are however several arguments against an assumption of an important role for the sympathetic nervous system in thyroid hormone-induced cardiac hypertrophy. Firstly although there are some contradictory results, most experimental work suggests that there is no increase in nervous activity f cardiac sympathetic nerves and no increase in cardiovascular sensitivity to catecholamines in hyperthyroidism (see review by Freedberg and Hamolsky 1974). Secondly it is possible to produce thyroxine-induced cardiac hypertrophy in reserpine-treated mice (Cohen *et al.* 1966). Thirdly as already mentioned above thyroxine has direct growth promoting effect on isolated myocardial cells in *in vitro* cell culture (see review by Greenberg *et al.* 1974).

The probable lack f an important role for the sympathetic nervous system in thyroid hormone-induced cardiac hypertrophy cannot be considered an argument against the proposed role for the sympathetic nervous system in *compensatory* cardiac hypertrophy as cardiac hypertrophy in hyperthyroidism could probably be regarded as pathological *developmental* hypertrophy rather than as true compensatory hypertrophy. Obviously the thyroid hormones themselves cannot be the final common pathway of compensatory cardiac hypertrophy as they induce generalized cardiac hypertrophy (Beznak and Korecky 1971) while compensatory cardiac hypertrophy is ften restricted to one side or even one chamber of the heart furthermore as already mentioned above the presence of thyroxine is not necessary for xercise-induced adaptive cardiac hypertrophy (Tipton and Tcheng, 1971).

Conclusions. I conclusion, therefore, t is suggested that increased nervous activity in cardiac sympathetic nerves induces compensatory growth of cardiac muscle cells, and that the trophic action is mediated by NA acting via β—receptor. It is furthermore suggested that in most or all conditions leading to adaptive cardiac hypertrophy the sympathetic nervous system is the final common pathway through which increased physiological or pathological demands for cardiac work trigger the biochemical events in the cardiac muscle cell resulting in adaptive growth.

Such an hypothesis carries some implications for clinical medicine. I animals cardiac hypertrophy can regress f the original growth stimulus is withdrawn (Hall *et al.* 1963 Van Liere *et al.* 1965; Leon and Bloor 1968 Beznak *t al.* 1969). Should the present hypothesis prove true it would open up possibd ties to influence the development f compensatory cardiac hypertrophy in patients with cond ions such as hypertension and valvular disease by using therapy which interferes with the activity f he sympathetic nervous system and it might be possible to prevent the hypertrophy from reaching the degree where cardiac performance begins to deteriorate. Furthermore the long term use of β—receptor agonists, in conditions such as atrio-ventricular conduction disturbances, and of non-selective β—agonists in asthma therapy might according to this hypothesis lead to an undesirable stimulus for

sessions the body weight remained the same as that of sedentary rats. As far as adaptive changes I the sympatho-adrenal system is concerned the intermittently trained rats showed values of noradrenaline content in heart and adrenal medulla that were intermediate between those of sedentary controls and those of rats subjected to daily xercise and, furthermore the excretion of noradrenaline and adrenaline in the urine following an exercise sessio was less than that of ntrained rats but greater than that f the daily-trained group.

5. Comparing physically trained rats with cold-acclimated rats the following main points emerged:

(a) Both groups developed the same degree of cardiac and adrenal hypertrophy but only cold-acclimated rats showed enlargement of the submandibular salivary glands.

(b) Cardiac noradrenaline content increased in both tra ned and cold-acclimated rats, but more so in the former group which was the nly group to show an increase n the concentration of noradrenaline in the heart. The noradrenaline content in the spleen was raised in the trained rats but decreased in the cold-acclimated group whereas only the cold-acclimated rats showed an increase in the noradrenaline content of the submandibular salivary glands. The content of noradrenaline in the adrenal medulla increased to the same xtent in both the xperimental groups, but only the trained group showed an increase in the adrenaline content of the adrenal medulla The noradrenaline content in the brain of cold-acclimated rats was unaltered in contrast to the increased values found in trained rats. It is suggested that these d fferences in adaptive responses reflect differences in the degree of activation of the various parts of the sympatho-adrenal system caused by the two experimental conditions studied.

(c) Trained as well as cold-acclimated rats excreted less noradrenaline in the urine during rest than controls did Following a session of swim-exercise only the trained not the cold-acclimated group displayed smaller increment in urinary noradrenaline excretion than that seen in sedentary warm-acclimated rats. After cold stress however smaller rise in the amount of noradrenalime excreted in the urine was found in both the trained and th cold-acclimated rats than in the control group

6 Inhibitio of noradrenaline synthesis following treatment with α-methyltyrosine leads to alterations in turnover rate about 4 hours after the treatment begins slowing I the turnover of noradrenaline occurs in the brain and an acceleration occurs the heart, during the 4 to 16 h period. Furthermore at the same time as the turnover in the brain slows down, the pre-existing difference in cardiac noradrenaline turnover between trained and untrained rats disappears. Thus, the inhibition of noradrenaline synthesis by α-methyltyrosine is completely unsuitable method for studying the normal physiological activity of noradrenergic neurons, both central and peripheral, at any time later than 4 hours fter administration of the drug.

7 I order to study the role of sympathetic nerves in adaptive cardiac hypertrophy rats were subjected t permanent chemical sympathectomy by daily injections of high doses of guanethidine (30–60 mg/kg BW) for si week

(a) This treatment led to an extensive destruction of sympathetic ganglion cells with a decrease of organ noradrenaline content f 90% the spleen and the submandibular glands and of 83% in the heart there was compensatory 45% increase in adrenal noradrenaline content. Guanethidine-treated rats at rest excreted 45% less noradrenal ne in the urine than the untreated rats, but this resting noradrenaline xcretion was by no means maximal since, fter xercise the guanethidine-treated rats increased their excretion of noradrenaline by xactly

the same proportion as the untreated rats did. It is suggested that the low level f nor adrenaline excretion by resting guanethidine-treated rats, in spite f their ability to xcrete more is reflection of the degree of denervation supersensitivity that develops in the vascular smooth muscle

(b) The guanethidine treatment caused reduction in weight gai and in the higher dose also an actual impairment of body growth as xpressed by final body length.

(c) Chemical sympathectomy with guanethidine treatment completely abolished the adaptive cardiac hypertrophy in rats subjected to daily xercise but did not abolish the cardiac hypertrophy induced by isoprenaline treatment.

MAIN CONCLUSIONS

The questions posed at the end of Chapter 1 can now be answered.

1 Daily physical exercise induces adaptive changes in both central and peripheral noradrenergic neurons in the rat. The stores of noradrenal ne are increased in brain, heart and adrenal gland, and at least transiently in the spleen as well. There are changes (nervous) activity in both central and peripheral noradrenergic neurons, but whereas the activity is *increased* in the brain, the activity f peripheral sympathetic neurons is *decreased* in both heart and spleen and probably in the vasculature as well. It is suggested that the decrease in activity in peripheral sympathetic neurons is secondary to an increase in inhibitory activity by central noradrenergic neurons. This central effect may in turn be at least partially reflex adjustment to the increased sensitivity to the vasoconstrictor actions of nor adrenaline that occurred in the trained rats.

2. The bradycardia of the "athlete's heart may be largely or entirely due t the decrease in cardiac sympathetic nervous activity caused by training.

3 Physical training appears to induce cross tolerance" to cold stress, while cold-acclimation does not lead to cross tolerance" to acute exercise.

4 The adaptive cardiac hypertrophy produced by chronic xercise is not caused by direct effect f the increased workload on the cardiac muscle cell, but is instead mediated by release of nor adrenaline from cardiac sympathetic nerves Furthermore ncreased activity of cardiac sympathetic nerves may be the final common pathway in all forms of compensatory cardiac hypertrophy

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Human Power at Subnormal Body Temperatures

by

Ulf Bergh

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FROM THE DEPARTMENT OF PHYSIOLOGY III
KAROLINSKA INSTITUTET
STOCKHOLM SWEDEN

Human Power at Subnormal Body Temperatures

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This thesis is based on the following papers

I NADEL E R HOLMÉR I BERGH U ASTRAND P -O & STOLWIJK J A J 1973 Thermoregulatory shivering during exercise Life Sci 13 983 989

II BERGH U EKBLOM B HOLMÉR I & GULLSTRAND L 1978 Body temperature response to a long distance swimming race Swimming Medicine IV (Eds B O Eriksson & B Furberg) pp 342 344 University Park Press Baltimore

III DAVIES M EKBLOM B BERGH U & KANSTRUP I L 1975 The effect of hypothermia on submaximal and maximal work performance Acta Physiol Scand 94 201 202

IV BERGH U HARTLEY H LANDSBERG L & EKBLOM B 1979 Plasma norepinephrine concentration during submaximal and maximal exercise at lowered skin and core temperatures Acta Physiol Scand 106 383 384

V BERGH U & EKBLOM B 1979 Physical performance and peak aerobic power at different body temperatures 1979 J Appl Physiol Respirat Environ Exercise Physiol 46 885-889

VI BERGH U & EKBLOM B 1979 Influence of muscle temperature on muscle strength and power output in human skeletal muscle Acta Physiol Scand 107 33 37

VII BERGH U BLOMSTRAND E & ESSÉN B 1980 Human anaerobic energy yield and muscle metabolites at subnormal body temperatures Manuscript

I addition some hitherto unpublished results will be presented

The papers are referred to in the text by their Roman numerals

C O N T E N T S

Abbreviations and symbols

The abbreviations and symbols used in the text and figures are with minor exceptions adapted to the guidelines devised by the Glossary Committee of the International Union of Physiological Sciences (1973a b) The units of the SI system are used

aBP	arterial blood pressure
ATP	adenosine triphosphate
a v difference	arterio-venous difference
^{o}C	degree Celsius (Centigrade)
CP	creatine phosphate
G-6 P	glucose-6-phosphate
HR	heart rate
peak HR	highest HR measured in a particular situation
J	joule
LA	lactate
NE	norepinephrine
peak oxygen uptake	highest oxygen uptake measured in a particular situation
maximal oxygen uptake; maximal aerobic power	highest oxygen uptake obtained irrespective of body temperature during exercise with large muscle groups breathing normal air at an air pressure equal to sea level
Q	cardiac output
RPE	rated perceived exertion
SV	stroke volume
T_c	core temperature
T_{es}	esophageal temperature
T_m	muscle temperature
T_{re}	rectal temperature
$\bar{T}_{sk}$	mean skin temperature
T_w	water temperature
W	watt
WT	work time
Ventilatory equivalent	pulmonary ventilation/liter of oxygen uptake

INTRODUCTION

Man is homeotherm which means that the body temperature is kept at a nearly constant level practically independent of the en vironmental temperature This enables man to maintain a high level of functional capacity in a great variety of environmental conditions though the metabolic cost of this privilege is rela tively high On the other hand man is unable to escape very far from homeothermy without approaching lethal body temperatures

A constant body temperature will be maintained only if heat production and heat gain are balanced by an equally large heat loss To ensure homeothermy in conditions involving a change in the ambient temperature and/or the level of physical activity one must therefore be able to change any of these variables con siderably This can be achieved in two distinctly different ways 1) physiological thermoregulatory responses such as increased metabolic rate (mainly by shivering) sweating and changes in the peripheral blood flow 2) behaviour e g clothing shelter external heating Behaviour has greatly extended the range of temperature in which man can survive and live as a permanent resident This refers mainly to cold since the physiological thermoregulation seems to be predominantly geared towards heat dissipation Man can be considered to be a tropical animal

Notwithstanding these powerful means of maintaining in dependence of the environmental temperature homeothermy is in fact only partial and furthermore it is limited as pointed out by Burton and Edholm (1955) Partial because many tissues e g skin and muscles display a considerable variation in temperature Actually only the body s central parts (the core i e CNS heart viscera) have an almost constant temperature The largest temperature gradients as well as the greatest temperature variation in a given tissue occur during cold exposure Homeo thermy is limited in that man cannot withstand the more severe cl mates o this planet without behaviou al responses Inasmuch as beha ioural protectio cannot always be ensured o sometine s even voluntarily abandoned man has to face the risk of losing the homeothermy battle with consequences that range from moderate hypo- or hyper thermia to lethal temperature levels Thus cold exposure can produce hypothermia of such a degree that many bod functions are seriously affected and it may even

cause death (for refs see Keatinge 1969 MacLean & Ernstie-Smith 1977) In these instances the exposure is usually accidental e g shipwrecks a fall from a boat rapid changes in weather e g during hikes in the mountains Voluntary exposure to less severe conditions is not unusual Activities involving water immersion are of special interest mainly because the rate of heat loss from the body can be very high in water

Thus it is of interest to know the body temperature response to such conditions Furthermore it is important to be aware of the price (in terms of the effect on optimal function) of deviations from homeothermy

Previous studies on humans have predominantly been concerned with resting conditions and/or submaximal exercise Few data are available on exhaustive exercise Furthermore most investigations have been conducted with the subjects immersed in water which has some inherited problems (cf Methodological aspects) Studies relevant to this topic will be discussed below in connection with the results of the present studies

Present knowledge in this field is limited concerning

1) the incidence and extent of body cooling during exposure to moderately cold (18–20°C) water

2) the possibilities of separating the evenutal effects of T_{es} T_m and T_{sk} on various factors during maximal exercise

3) the possibilities of explaining the observed effects of body temperature on HR oxygen uptake and performance

4) quantifying the effects of changes in various body temperature on functions of importance for the physical performance

Thus the object of the present series of experiments was to extend present knowledge of the abovementioned problems at a range of subnormal body temperatures where there are no symptoms of severe hypothermia and/or frostbite

METHODOLOGICAL ASPECTS

Most previous studies have been performed with the subjects immersed in water of different temperatures which has some definite drawbacks In water there is often a co-variation between T_c and T_{sk} making it difficult to seperate their relative importance As the temperature of water affects its physical properties external work might change which would complicate the measurements of performance Swimming is technically rather difficult and distal parts cool more quickly than the rest of the body Those two factors together might induce a limitation to performance before other factors are stressed to their maxima Furthermore swimming does not induce a maximal load on aerobic power (Holmér 1972)

Therefore in order to reduce these problems in the present series of experiments it was considered necessary to

1) use technically uncomplicated types of exercise
2) use water only as a cooling medium and to perform the experiments in air
3) use combined arm- and leg exercise when the object was to stress the aerobic power to its maximum (cf Bergh et al 1976)

Low body temperatures were produced by immersion in cold water either the whole body (I III IV V) or just the legs (VI VII) Different levels of skin temperatures were achieved by regulating the ambient temperature at suitable levels sometimes in combination with exposure of the subject to increased air velocity and artificial skin wetness

Methods

Only a summary of the methods used is given here For details the reader is referred to the separate reports

Bicycle exercise was performed in an upright position on mechanically b aked ergometers (Monark) either as leg exercise (I VI VII) or as combined arm and leg exercise (III IV V) In the latter case the ratio between arm and leg work rates was 1/4 which produces the same maximal oxygen uptake as treadmill running (uphill) (Bergh et al 1976)

The exhaustive exercise tests on a bicycle ergometer were performed for 1 5 8 min at a constant pedal rate (60 revolutions min $^{-1}$) and the maximum work time was used as a measure of physical performance The point of exhaustion was considered to have been reached when the subject was unable to keep up the pace for 5 consecutive revolutions (III IV V VII)

Oxygen uptake was calculated from expired air samples collected in Douglas bags Volume was measured in a balanced spirometer Gas samples were analyzed for CO_2 and O_2 content by the Haldane or the Sholander technique (I III) or by the use of a mass spectrometer (MGA 200 UK) (Hallbäck et al 1978) (IV V VII)

Oxygen deficit was derived by subtracting the total oxygen uptake from the calculated oxygen requirement of the actual rate of work (Astrand & Rodahl 1977) (VII)

Heart rate was calculated from ECG-records

Blood lactate concentrations were determined by the Baker Summerson method as modified by Ström (1949)(III IV V VII)

Arterial blood pressure was measured by a sphygmomanometer (IV)

Muscle biopsies were obtained with the percutaneous needle biopsy technique (Bergström 1962) (VI VII)

Muscle samples were analyzed with methods described by Essén (1978) (VI VII)

Plasma norepinephrine concentration was measured as described by Lake et al (1978) (IV)

Maximal muscle strength in the knee extensors was measured on an isokinetic dynamometer (Cybex II Lumex Inc New York)(VI)

Sprinting performance was defined as the shortest time in which 20 revolutions (8 5 kJ) could be performed on a bicycle ergometer (VI)

Jump ng performance was evaluated by measuring the height of a vertical jump (VI)

The subjective feeling of exertion was rated according to a scale desc ibed by Borg (1974) (III IV V VII)

Body temperatures have been measured by thermocouples except in study II where mercury rectal thermometers were used core temperat e was continuously measured in the esophagus at the level of the heart muscle temperature in the vastus

lateralis muscle Mean skin temperature was calculated from measurements made at 6 8 different skin locations (Saltin et al 1968) The recording equipment and each sensor were cali brated at each individual trial

Th errors of the methods for the determination of variables are pre nted in Table 1

Subjects

In most of these studies only male subjects participated They were all physically well trained and familiar with the labora tory procedures Only in study II both male and female subjects took part The subjects were informed about all aspects of the experiments before they consented to participate

Procedure

To avoid the influence of systematic errors and possible training effects the order of the experimental situations was varied at random and a maximum of two experiments was performed per week with each subject

Statistics

Conventional statistical methods were used to calculate mean values standard deviations and correlation coefficients The t test was applied to analyze the significance of differences The error of the method has been specified as the coefficient of variation (c v) of a single value and has been calculated from the following formula

$$c\ v \quad \sqrt{\frac{1}{2} \quad \frac{\Sigma (d \quad \bar{d})^2}{n \quad 1}} \quad \frac{1}{\bar{x}} \quad 100$$

where d is the difference between double values d is the mean difference n is the number of determinations and x is the mean value

Comments

The vast s lateralis muscle was chosen for T_m measurements and fo obtaining muscle biopsies since this muscle is highly active dur g the type of exercise performed in these experiments (Hen riksson and Bonde-Pedersen 1974) and the risk of complications is minimal

T_m was measured immediately after exercise The values presented are peak values obtained at a depth of 30 50 mm from the skin surface In experiments where both T_m and muscle biopsies were obtained the measurements were made as close to each other as possible (within 1 cm) In some of the present investigations attempts were made to quantify the effects of changes in body temperatures In this context one faces the problem of evaluating the representativity of various temperature measurements In the case of core temperature it was considered necessary to find a site of measurement which reflects the temperature both of the CNS and of the heart This is essential when trying to relate both thermoregulatory and circulatory responses to internal temperature The choice of esophageal temperature as the measure of core temperature in the present studies was dictated by two main considerations 1) T_{es} responds faster than rectal temperature during the temperature transients of the exercise which might be important during heavy exercise of 3 8 min duration Thus rectal temperature will underestimate heart and brain temperature if these are increasing 2) Also tympanic temperature has a fast response but the measurements are usually rather unpleasant during heavy exercise since the sensor tends to irritate the tympanic membranes as a result of the large ventilation and the body movements

A teflon esophageal probe is usually tolerated very well by the subjects both at rest and during exercise irrespective of the latter s severity

A more difficult problem is to judge the representativity of a T_m measurement The present investigations used the peak T_m values obtained at 30 50 mm from the skin surface The primary reason was that it is easier to obtain reproducible values with this procedure compared to temperature measurements at a fixed depth Anothe reason was that in an exercising muscle the highest value is likely to occur in the muscle s most active parts and these areas might be of special importance with regard to physical performance On the other hand other parts of the muscle evidently contribute to total tension development Hence it is not really possible to judge whether it is more relevant to relate the various changes to peak T_m or to average T_m In the latter case one has to keep in mind that during cooling the

temperature gradients in the muscle are higher (Pugh 1967) Thus a T_m measurement at a point with above-average T_m will overestimate the temperature effect on various variables Another problem is the above mentioned temperature transients which makes it more difficult to relate different variables to temperature In order to minimize these problems temperature and other recordings were made as close to each other as possible (30 s) However the measurement of the total oxygen uptake (and oxygen deficit) is the exception from this since T_m was only measured before and after the exercise

Table 1 Error of the method for some variables measured under different conditions expressed as the coefficient of variation

Esophageal temperature	rest (15 min) separate days	0 3 %
	after 5 min of maximal exercise separate days	0 1 %
Muscle temperature	rest (15 min) separate days	1 4 %
	after maximal exercise separate days	0 5 %
Peak torque		
0^{o} $^{o}/s$	same day	4 5 %
90^{o} $^{o}/s$		4 9 %
180^{o} $^{o}/s$		6 7 %
0^{o} $^{o}/s$	different days highest value of 2 trials	6 1 %
90^{o} $^{o}/s$	different days highest value of 2 trials	6 5 %
180^{o} $^{o}/s$	different days highest value of 2 trials	7 0 %
Vertical jump	same day	3 5 %
	different days highest value of 2 trials	1 5 %
Maximal bicycling x)		
2 5 3s	separate days	2 4 %
10 15s		2 2 %
ATP CP G-6 P LA concentrations	see Essén 1978	
Oxygen uptake		
submaximal exercise		1 5 %
maximal exercise		2 1 %

x) speed

RESULTS AND DISCUSSIONS

This section is divided into five parts

Rectal temperature after long distance swimming (II)
Aerobic power (I III IV V VII)
Anaerobic power (VI VII)
Maximal muscular strength (VI)
Physical performance (I II III IV V VI VII)

Rectal temperature after long distance swimming
The subjects were 8 females and 41 males of various ages (13 63 years) and training status (200 70 000 m of swimming per week) Only 15 of them were competitive swimmers The time needed to finish the 3 2 km race ranged from 37 to 150 min (mean 72 min) Mean T_{re} before and after the race was 37 7 °C (n 34) and 36 4 °C (n 49) respectively The comparatively high T_{re} at the start was due to warm up activities e g jogging calisthenics

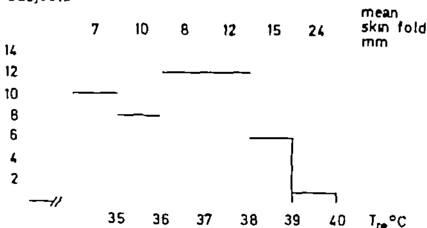


Fig 1 The numbe f subject who ttained ch level f r ct l tempe t re (T_{re}) t the end of 3 2 km swimmi g race (5 f the partl ipants did t fi i h) Th numbers i the upper part f the figur den t ave ge mean ki f ld v lue f th subj t i h f the T_{re} i t rval The w t r tempe ture was 19 °C (II)

Despite this increased heat content of the body only 19 of the subjects had a T_{re} above 37 °C at the finish thus the majority of the subjects had more or less subnormal T_{re} (Fig 1) and 10 of them reached a T_{re} below 35 °C Furthermore 5 of the participants were unable to complete the race and had to be helped out of the water Their symptoms included shivering and cramps and in some cases dizziness In contrast some subjects increased their T_{re} during the race

The difference in T_{re} response was related both to skinfold thickness (cf Pugh & Edholm 1955 Keatinge 1969 Hadel et al 1974 Holmér & Bergh 1974a Boutelier et al 1977 Golden et al 1979) and to exposure of time the former seemed to be the more important one

Evidently exposure of this type can produce subnormal body temperatures in most subjects

Aerobic power

Rest and submaximal exercise At T_{es} lower than 36 °C oxygen uptake was increased above normal levels (I III IV)

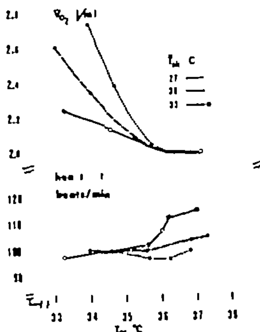


Fig 2 Oxyg n pt ke and he rt rat i on subj ct d ri g submaximal e er i (150 W) t diff r nt c re (T_{es}) and mean skin tempe at r (T k) (F om Be gh & Ekblom 1978)

The magnitude of this increase was inversely related both to T_{sk} (Fig 2) and to T_{es} (I) There are also data indicating an inverse relationship between the magnitude of the extra oxygen uptake and the rate of work (Fig 3) However the latter finding was not consistent

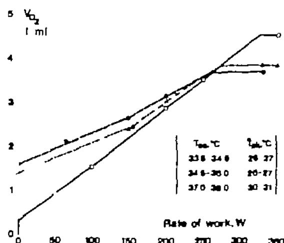


Fig 3 Oxygen uptake as function f the t f work at rmal and lowe ed body temperatur Not the levelling ff t th high t s f work (Ave ag v lue = 3)

The extra oxygen uptake can be a result of shivering non-shivering thermogenesis or lowered mechanical efficiency during exercise The major part of this extra energy yield is most likely an effect of shivering at least at rest where no work is per formed and there are little evidence that non shivering thermogenesis contributes substantially to an increase in metabolic rate during acute cold exposure There are reasons to believe that temperature can influence mechanical efficiency (e g by affecting nerve conductivity shivering interfering with co-ordination) However the present data do not support such an assumption T_{es} could be changed considerably (from 37 to 35 °C) without any substantial effect on the metabolic rate (Fig 2)

As mentioned earlier the magnitude of extra metabolic rate at subnormal T_{es} was affected by both T_{es} and T_{sk} indicating that shivering can be elicited by central as well as by peripheral cold stimuli The latter seems to be more important (Benzinger et al 1963 Nielsen 1976) Another factor that might influence the magnitude of the extra metabolic rate is the rate at which work is performed There are studies which indicate that the magnitude of the extra oxygen uptake is independent of the rate of work (Nadel et al 1974 Nielsen 1976 I) but other data suggest an inverse relationship (Craig & Dvorak 1968 Hong & Nadel 1979 and Fig 3)

The discrepancy between these results is partly of methodological origin Nielsen studied a rather narrow range of metabolic rates which did not induce any substantial differences in the extra metabolic rate As Nadel et al (1974) did not examine the different levels of work intensities at equal body temperatures it is not possible to tell whether they induced any differences It is however reasonable to believe that work intensity could influence the magnitude of shivering at least at higher rates of shivering involving a relatively large muscle mass Hence as the rate of work increases more and more of the shivering muscle mass becomes involved in exercise and the magnitude of shivering would then decrease provided other muscles do not compensate by increasing their shivering activity The purpose of shivering is to generate heat Teleologically an increased rate of work (increased heat production) would diminish the need for shivering A decrease in the magnitude of shivering would be a logical consequence even for muscles which are not voluntarily activated In fact Hong & Nadel (1979) found that shivering activity in the sternocleidomastoideus muscle of the neck was suppressed during bicycling and more so the heavier the work on the ergometer

The cold induced increase in oxygen uptake was usually not accompanied by an elevation of heart rate which was unchanged or even reduced at rest (IV) and at a given rate of work (I III) (cf Fig 2) This indicates that there must have been a change in either cardiac output stroke volume and/or arterio-venous O_2 difference McArdle et al (1976) reported that $\dot{Q}$ and a $\bar{v}O_2$ difference were unchanged while SV at a given oxygen uptake was

higher during exercise in 18 °C water compared to 33 °C water Thus at a given rate of work Q and a $\dot{V}O_2$ difference must have been higher in 18 °C Their data are unfortunately not possible to relate to core temperature

The reduced HR at a given oxygen uptake cannot be explained by a diminished sympathetic activity for the plasma norepinephrine concentration was higher at low body temperatures (IV Fig 4)

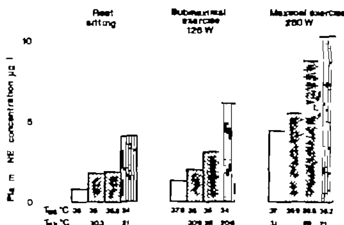


Fig 4 Plasma norepinephrine (NE) concent tion t re t submaximal and maximal exe i t diff rent combi tion of esophageal (T_{es}) and mean kin temperatur (T_{sk}) Not that both low T and low T_{sk} ca levat th NE-concentr tion and that the ff t seem to be additive (Averag v lue n 2; IV)

However the possibility still exists that β-receptor activity is reduced which is supported by the fact that β receptor blockade did not further reduce HR during exercise in hypothermia (III) in contrast to the reaction at normal body temperature (Ekblom et al 1972) It is also possible that peripheral vasoconstriction enhances venous return and thereby increases SV Furthermore vasoconstriction can induce an elevated aBP and thereby elicit an increased activity in the pressor receptors which might depress HR in order to normalize aBP At rest (sitting) aBP was higher at subnormal T_{es} and/or T_{sk} while during submaximal exercise aBP was the same or lower (IV) Furthermore

during submaximal exercise aBP was lowered in relation to oxygen uptake and HR at low T_{es} plus normal $\bar{T}_{sk}$ but not at normal T_{es} plus low T_{sk} These findings may indicate that the observed depression of HR is due to a combined effect of a pressor receptor induced HR decrement and a reduced β receptor activity (IV)

One important implication of this HR response to lowered body temperatures is that predictions of physical work capacity on the basis of HR measurements during submaximal exercise will be misleading Thus measures such as PWC_{170} watt pulse and oxygen pulse will lead to an overestimation of physical work capacity If the estimation is based on measurements of HR at a given oxygen uptake the overestimation will be even greater than if the calculation is made from HR and rate of work since oxygen uptake at a given rate of work is increased at subnormal T_{es} without a concomitant rise in HR

Pulmonary ventilation at rest and during submaximal exercise increased with decreasing T_{es} and $\bar{T}_{sk}$ while the ventilatory equivalent was elevated only when a low T_{es} was combined with a low T_{sk} (unpublished results) This indicates that a cold skin constitutes an additional drive on the ventilatory center superimposed on the drive from the metabolic demand

Exhaustive exercise Peak oxygen uptake was positively related to T_{es} (r 0 88) and T_m (r 0 91) (V Fig 5) The rate of these changes was approximately 5 -6 % $^{o}C^{-1}$ Similar results have been obtained during swimming (Holmér & Bergh 1974b) No subject was able to attain his control maximal oxygen uptake at T_{es} and T_m lower than 37 5 and 38 0 °C respectively (V Fig 6) This response to variation in T_{es} and T_m was unaltered by a moderate change in T_{sk} (cf Fig 6 & 7) However it should be kept in mind that T_{sk} will sooner or later affect T_{es} and T_m (cf swimming in cold water) and thereby cause considerable indirect effects on different variables (even if their dependence of T_{es} and T_m is not modified directly)

Lower T_{es} and T_m did not affect oxygen uptake response during the first two minutes of exercise (V VII) Thereafter oxygen uptake was related to T_{es} and T_m i e at normal temperatures oxygen uptake continued to increase significantly while

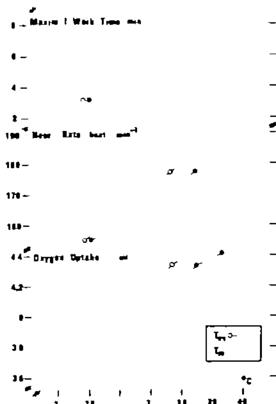


Fig 5 A ag v lue (8) for time to exhaustion peak he rt t and peak oxygen uptak n rel tion t esophag l (T_{es}) and va us l t lis empe ture (T_m) (V)

it levelled off at subnormal T_{es} and T_m in spite of a constant rate of work

Peak HR was related to T_{es} and T_m in the same manner as peak o ygen uptake (V Fig 5 & 7) which is in accordance with data obtained during swimming (Nadel et al 1974 Holmér & Bergh 1974a) The difference between the highest HR attained by each subject (regardless of temperature) and the HR peak at respective T_{es} and T_m was correlated both to T_{es} and to T_m (r 0 97 and 0 95 respectively) and the effect of temperature on peak HR amounted to 8 beats min ^{1} ^{o}C ^{1}

The data on HR during the first min of exercise are contra dictory in two studies HR was unaltered by T_{es} and T_m (V) and parasympathetic blockade This discrepancy might originate from the fact that lower relative work rates were performed in study III and V than in study VII

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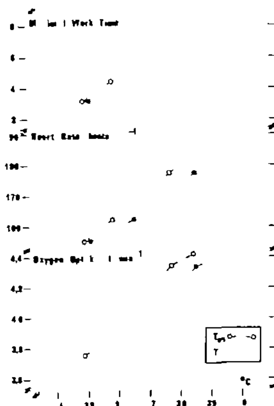


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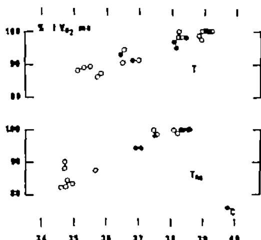


Fig 6 P k oxyg n uptake at the end of xhau ti e erci f r 3-8 mi in per cent of ea h bje t maximal oxyg n uptake b- tained at 2 level f mean kin temperature (T_{sk}) in r lation t diff r t oph g l (T_{es} lower panel) and vastus lateralis (T_m upper pan l) temperat es Filled ci le $\bar{T}_k$ 27 °C pen ci l s $\bar{T}_k$ 31 °C (Data on 8 s bj t ; V)

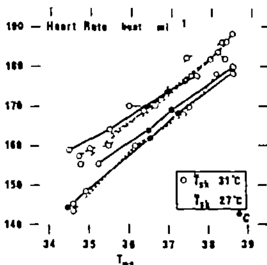


Fig 7 P k ho t ate t the end of xhaustive rci t 2 levels of mean ki tempe t (T_{sk}) (8 V)

What effect of lowered T_{es} and/or T_m can cause the decrement in peak oxygen uptake? The shorter work time may of course prevent oxygen uptake from accelerating to its maximum potential However at the first two min of exercise (V and VII) oxygen

uptake was the same irrespective of temperature but it levelled off at a lower value at subnormal T_{es} and T_m compared to normal or elevated body temperatures (V) Neither can a relative hypoventilation explain the reduction in peak oxygen uptake since the pulmonary ventilation was the same and the ventilatory equivalent was higher at low body temperatures (V) Oxygen diffusion rate is of course related to temperature but hardly to an extent that would induce a measureable change in peak oxygen uptake within the temperature range used in the present study The most conspicuous finding in relation to the decrement in peak oxygen uptake is the lowered peak HR especially since the change in peak HR was well related to the change in peak oxygen uptake (r 0 78)

Temperature may affect HR in many ways e g 1) by a modification of the activity in the autonomous nervous system 2) by causing a reflex activity from the pressor receptors 3) by affecting the heart muscle (its ability to produce tension) Since atropin did not alter this HR response (III) an increased para sympathetic activity is not a likely explanation for the reduction in peak HR Neither can a decreased sympathetic activity serve as a plausible explanation for lowered body temperatures were accompanied by greatly increased plasma NE concentrations (IV) However β receptor activity might still be decreased by lowered tissue temperature A reflex activity from pressor-receptors as a mediator of a HR reduction is less probable considering that aBP during maximal exercise was lower at lowered T_{es} (IV)

The present data accordingly indicate that the decrement in peak HR can result from a direct effect of temperature on the heart muscle Thus the speed of contraction of any muscle is dependent on the load (force) against which it is contracting An increase in the resisting force will inevitably result in a decrease in the maximal speed of contraction Thus for a given stroke volume and blood pressure (afterload) there is a maximal speed of contraction which cannot be exceeded A lowered tissue temperature will induce a leftward shift in the force velocity relationship (at a given force the maximal velocity is decreased) Thus peak HR should theoretically decrease if the temperature of the heart muscle is lowered A direct effect of

temperature on the heart muscle is indicated by the facts that at low tissue temperature 1) there was an *increase in the time* necessary to reach peak tension and a reduction in the tension increment per unit of time in isolated heart muscle preparations (Ekblom & Bing 1979) 2) the effect of temperature on peak HR was of the same order of magnitude as that in maximum muscle strength observed in skeletal muscles (VI) Two main possibilities are available for explaining the reduction in peak HR 1) decreased β receptor activity 2) reduced force development The observed reduction in aBP may be a consequence of a reduced cardiac per formance

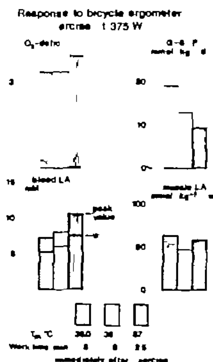
Anaerobic power

The immediate source of energy for muscle contraction is ATP The normal ATP stores are however consumed by a few maximal contractions A prolongation of muscular exercise necessitates *resynthesis of ATP Anaerobically* ATP can be produced via a breakdown of CP and by glycogenolysis and glycolysis The stores of CP can be depleted in less than 10 s (Karlsson 1971) while the stores of glycogen are much larger and are not normally emptied by exhaustive exercise of 2 3 min duration The maximal rate at which energy is delivered through these various proces ses differs For these reasons it is necessary to study power output during exercise of varying duration in order to investi gate the effect of temperature on the different candidates yielding energy It should be emphasized that these experiments were carried out after local cooling of the legs Thus there was little or no shivering

For a single muscle contraction (knee extension), peak power output was *positively related to temperature The magnitude of* change was 4 6 $^{o}C^{1}$ (VI) Average power output during the first revolutions of maximal bicycling and the height of a maximal vertical jump (VI) were affected qualitatively and quantitatively in the same way as peak power output during knee extensions These findings cannot be explained by differences in the quantities of available ATP since its concentration was unaffected by T_m (Edwards et al 1972 VII) Thus it is more likely that tissue temperature affects the rate of ATP breakdown and/or the *transformation from chemical energy to mechanical work*

At a maximal effort lasting 5 to 10 s e g maximal bi cycling CP breakdown is the main anaerobic source for ATP resynthesis and thus energy yield In this type of exercise power output changed as described for peak power and during knee extensions (VI) *This must mean that ATP production by CP-break* down was not more affected by lowering of T_m than was ATP break down and/or that the latter step is the rate limiter

When exercise is performed at a rate which leads to ex haustion in 1 to 3 min the major part of the anaerobic energy is derived through glycogenolysis (Karlsson & Saltin 1970) In this type of exercise (VII) time to exhaustion was shorter at subnormal T_m Average values were 1 8 min at T_m 35.0 °C and 2 5 min at T_m 37 1 °C Oxygen deficit and blood LA-concentration (both at exhaustion and the peak value) were lower while muscle *LA-concentration was somewhat higher at low T_m (Fig 8) The*



F g 8 Oxygen defi it and the cha ge (Δ) i glucose-6-phos phat blood and mus l concentr tions f l ct t (LA) d ing bi y l xe ise (375 W) t diff t vast l t rall tempe tur (T_m) and work time Not that the subject we exhausted ft 1 8 min t low T_m whil the same work rat wa su t ined f 2 5 min t normal T_m (n 4; VII)

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rate of LA removal as indicated by the lower rate of blood LA increase during exercise at low T_m (this can be induced by a reduced flow lower diffusion rate and/or decreased LDH-activity) But it is also conceivable that the O_2-deficit calculated from oxygen uptake measured over the lungs does not mirror oxygen uptake over the working muscles in the same way at different body temperature levels An O_2 deficit in these muscles can be underestimated if there is an elevated oxygen uptake in other tissues e g as a result of shivering This is probably not the case in the present experiments since oxygen uptake at rest immediately before the start of exercise was the same in all experiments performedafter local cooling of the legs β receptor blockade can decrease LA removal from an exercising muscle (Juhlin-Dannfeldt & Aström 1979) i e a similar event as noted in the low T_m experiments As discussed on p 19 a reduced T_{es} may suppress the β-receptor activity

Muscular strength

Maximal isometric strength was little affected by T_m (2 % $^{o}C^{-1}$) whereas T_m did have a considerable effect on maximal dynamic strength at all studied speeds of contraction (4 5 % $^{o}C^{-1}$) Thus the force velocity curve was shifted to the left (Fig 9) The present results on maximal muscle strength are in agreement with previous studies in humans (Asmussen et al 1976 Binkhorst et al 1977 For refs see Falls 1972) In contrast Asmussen et al (1976) demonstrated that during eccentric contraction the capability to store tension (produced e g by a downward jump) is enhanced by a decrement in T_m This is further supported by the finding that the ability to perform eccentric bicycle exercise was lowered at very high T_m (Nadel et al 1972) Furthermore endurance in isometric exercise is reported to be enhanced at subnormal T_m Thus the influence of temperature on muscle strength is d fferent in different types of exercise The cause of this phenomenon is still unknown Asmussen et al (1976) suggested that temperature influences the rate of breaking and formation of the cross bridges between the actin and the myosin f laments The above-mentioned results fit this hypothesis very well since s ch a mechanism would involve a resistance to any change n muscle length Thus in eccentric and in isometric

endurance exercise the resistance will enhance external force development and vice versa in concentric exercise Maximal isometric strength should consequently be unaffected by temperature However an isometric contraction usually has in initial shortening phase in regard to the muscle filament A small negative influence on force development would then be expected and has in fact been reported (Asmussen et al 1976 VI)

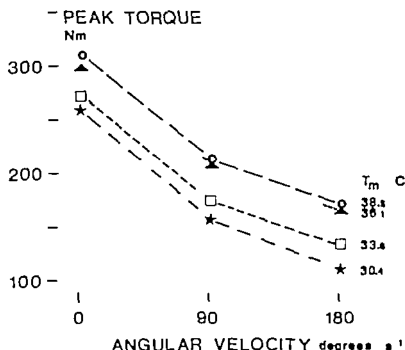


Fig 9 Peak t rque as function f g lar locity f the knee j int during t nsion (th f e-velocity l tionship) t diff t l el f T_m (A g l 4 VI)

It is also interesting to note that the temperature effect on power was of the same order of magnitude over a wide range of peed of movement (measured during bicycling VI)

Physical performance

Physical performance is in a broad sense a function of energy output neuromuscular function and ps chological factors The present series of e periments concentrated primarily on the

first of these factors However some information about the other two has also been obtained

Performance was found to be decreased at subnormal temperatures in exercises consisting of one up to several hundred contractions This is not surprising since factors affecting the energy yield (both aerobic and anaerobic) were negatively influenced by a lowered tissue temperature In exercise lasting longer than 2 min the major part of the energy demand is covered by aerobic processes Therefore the observed decrement in peak oxygen uptake will have an adverse effect on performance in such types of exercise If performance is expressed as $W_{max}6$ (i e a prediction of the rate of work that can be performed during 6 min based on maximal work time for any exhaustive rate of work see Tornvall 1963) the change corresponded to approximately 8 % $^{o}C^{-1}$ for exercises lasting 3-8 min

In exhaustive exercise for less than 2 min the change in $W_{max}6$ was 5-6 % $^{o}C^{-1}$ which is of the same order of magnitude as the difference in maximal power output in 1 20 contractions The obvious explanation for the greater effect of body temperature on performance in the former type of exercise is that there is a change in both aerobic and anaerobic energy yield in contrast to short time exercise where oxygen uptake at a given work time was independent of temperature

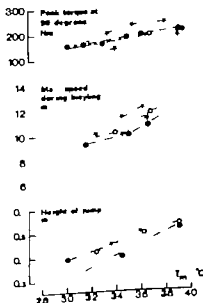


Fig 10 Indi idual d t f peak t rque d ing knee x- ten ion maximal pe d f the fly-wheel d ring bicycling f 10-15 and height f ve ti l jump t diff ent vasto l t li tempe t (T_m) (4; VI)

Performance in the sprinting and jumping tests was positively related to T_m (Fig 10) The decrements were proportional to the changes in peak torque (maximal dynamic strength) thus indicating that factors such as coordination were little affected One must however bear in mind that the jumping and sprinting tests consisted of rather uncomplicated movements (stationary bicycling and vertical jumping) which require fairly little skill Furthermore the experiments were carried out at ambient temperatures of 22 24 °C after local cooling of the legs which induced quite small effects on T_{es} and T_{sk} thus resulting in minimal shivering Severe shivering might interfere with an optimal coordination and thereby cause a further decrement in performance without a concomitant decrement in maximal muscle strength (unpublished results) Very low T_{sk} will also inhibit sensory impulses from skin receptors for pressure and touch which might result in very severe disturbance in the motor function

The increased metabolic rate for a given task will of course lead to a faster depletion of energy stores of which glycogen and glucose are the more critical for prolonged exercise (especially when this leads to hypoglycemia which may affect thermoregulation) Alcohol consumption in combination with reduced liver glycogen stores can elicit hypoglycemia and thus indirectly cause a fall in body temperature in individuals exposed to a cold environment (Haight & Keatinge 1973)

The energy cost (oxygen uptake) at a given rate of work is higher and the peak oxygen uptake is lower at lower body temperature and hence a reduced energy pool is available for physical work This indicates a greater relative strain on the individual It is however of interest to note that the subjects do not seem to have perceived any augmented feeling of fatigue they did not rate their perceived exertion (RPE) any higher at a given rate of work given oxygen uptake or given HR at lower body temperatures (Fig 11) The tendency is rather the opposite thus indicating that the subjects are unaware of the increase in the relative rate of work and the reduced potential for heavy exercise

During swimming in cold water however RPE was highe at given values for submaximal speed and HR (Fig 11) than in warm

water This discrepancy between the different types of exercise can result from a more pronounced cooling of the arms in combination with their greater relative involvement during swimming compared to bicycling It should be emphasized that tissue temperature mostly decreases during swimming in cold water while during bicycling at room temperature the opposite occurs Thus in bicycling the feeling of cold stress decreases gradually while it increases during swimming These observations indicate that the normal perception of exertion in relation to the rate of work is considerably altered by changes in temperature stimuli a fact that might be of importance in many situations

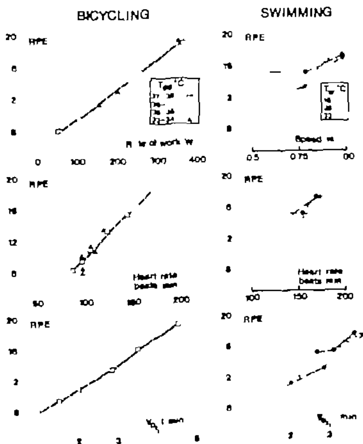


Fig 11 R ted pe lved ex rtion (RPE) i l tion t exe ise i ten iti (upper panel) hea t t and oxygen uptak (lowe panel) c diff ent sophag l temperat re (bicycling) and wat temper t re(T_w) (swimming)

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SUMMARY

Data are presented from studies analyzing the following

- The incidence and extent of body cooling during a 3 2 km swimming race in water at 19°C
- The effect of body temperatures on
  - aerobic power at rest and during submaximal and maximal exercise
  - anaerobic power at maximal efforts ranging from fractions of a second to 3 min
  - maximal muscle strength at different speeds of movement
  - physical performance evaluated in the following ways
  - Work time 1 5 8 min time to exhaustion during a standard maximal rate of work (bicycling) from which W_{max} 6 was calculated
  - Work time 1 15 s the velocity (of the fly-wheel) attained at maximal bicycle ergometer exercise with a given resistance (torque)
  - Work time < 1 s height of a vertical jump

1 Swimming 3 2 km in water at 19°C produced a subnormal core temperature in the majority of the participants

2 At rest and during submaximal exercise lowered esophageal (T_{es}) and mean skin (T_{sk}) tempe ature induced an increase in oxygen uptake above normal levels This increase was attr buted to shivering which was inversely related to T_{sk} T_{es} and less consistently to the rate of work

3 Heart rate (HR) was lowe at a given oxygen uptake at rest and during submaximal exercise at subnormal T_{es} and/or T_{sk}

4 Peak aerobic power du ing exhaustive exercise of 3 8 min duration was positively related to T_{es} and T_m (muscle temperature) (r 0 89 and 0 91 respectively) These relations were unaltered by moderate changes in T_{sk}

5 Peak HR was related to T_{es} T_m and T_{sk} in the same way as oxygen uptake The correlation between changes in HR and changes in oxygen uptake was 0 78

6 The calculated maximal anaerobic power was positively related to T_m in exercises lasting from 0 5 s to 3 min The effect of temperature was 4-6 % ^{o}C ^{1}

7 At work time < 2 min low T_m induced a faster rate of LA accumulation in the working muscle probably attributable to a lower rate of LA removal (LA lactic acid) At work time 3 8 min this was combined with a reduced aerobic power

8 ATP and CP concentrations in the muscle showed no consistent relation to T_m either at rest or immediately after exercise

9 Maximal muscle strength was positively related to T_m This effect of temperature amounted to 2 % ^{o}C ^{1} and 4 6 % ^{o}C ^{1} in isometric and dynamic exercise respectively

10 Physical performance was positively related to T_{es} and T_M The temperature effect amounted to 4 6 % ^{o}C ^{1} in maximal exercise of less than 3 min duration and 8 % ^{o}C ^{1} at 3 8 min exercise

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SYMPOSIUM ON THE EFFECTS OF NICOTINE ON NERVOUS FUNCTIONS

Edited by

THE MEDICAL ADVISORY BOARD OF
THE SWEDISH TOBACCO COMPANY
Stockholm Sweden

Stockholm 1980

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Symposium on the Effects of Nicotine on Nervous Functions

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Contents

Introduction

The Medical Advisory Board of the Swedish Tobacco Company was formed in 1957 in consultation with the National Board of Health The main task of the Board is to stimulate medical biological and chemical research into the effects of the use of tobacco and to advise the Company in sponsoring such activity in Sweden.

Over the years the Company has granted financial support to numerous research projects the results of which have been published in the scientific literature Three international symposia were held on the initiative of the Board with the aim of improving international exchange in this field of research

During the last few years there has been a noticeable increase in research activity aimed at elucidating how nicotine or smoking interacts with nervous mechanisms The Board therefore took the initiative of arranging a mini-symposium on the effects of nicotine on nervous functions held in Stockholm on 29 November 1978 The participants were recipients of grants from the Swedish Tobacco Company engaged in this type of research at first hand but in order to cover the subject more completely a few guest lecturers were invited. Thus Dr Boleslaw Srebro of the University of Bergen Norway made a survey of central cholinergic pathways and Dr Edith Heilbronn from the National Defence Research Laboratories Stockholm gave an outline of cholinergic receptors. The participants expressed the desire to make the symposium accessible to an international public by publishing the proceedings in English The Swedish Tobacco Company acknowledges with gratitude the co-operation of its grantees and their co-workers towards the production of this publication

L. Hjern
Secretary of the Medical Advisory Board of the Swedish Tobacco Company

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Assessment of cholinergic neuronal activity in the brain

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ABSTRACT Endogenous acetylcholine (ACh) ACh turnover and high affinity choline uptake (H.A. Ch uptake) have all been considered valuable markers for cholinergic neural activity The present communication compares the markers regarding regional differences in the brain of normal mice as well as in mice treated with oxotremorine (OT) nicotine and sodium pentobarbital. A relationship is obtained between in vivo turnover values for 5 different brain regions and in vitro H.A Ch uptake. Pretreatment with OT and sodium pentobarbital produces specific regional changes in both turnover and H.A. Ch uptake. However both drugs produce shifts of the correlation lines due to uptake being less affected than turnover Atropine completely prevented the effects of OT regarding turnover and H.A. Ch uptake. A convulsant dose of nicotine did not produce significant changes in any of the brain regions studied neither on turnover nor on H.A. Ch uptake.

Measurements of the activity of acetylcholinesterase (AChE) choline acetyltransferase (CAT) and *of the transmittor acetylcholine* (ACh) have been extremely valuable for the identification and investigation of cholinergic synapses. Especially the sensitive histochemical and microchemical methods for AChE have made it possible to obtain a very detailed picture of the regional distribution of cholinergic and/or cholinoceptive neurons in the brain.

Used together with cell fractionation technology the methods quoted have given a fairly good idea *not only about the localization* but also the organization of cholinergic synapses in brain Less information has been obtained regarding the dynamic aspects of cholinergic neuronal activity

Since the early discovery (Richter & Crossland 1949) that anesthesia and natural sleep produce inverse changes of the steady state level of acetyl choline (ACh) in the brain steady state levels have been the most commonly used indicator of cholinergic neuronal activity (Giarman & Pepeu 1962) (Table 1)

Due to the limitation of steady state le els other possibilities have been in estigated e.g release of ACh from the exposed cortex or from the inner surfaces of the brain (MacIntosh & Oborin 1953 Beani et al 1968 Hemsworth & Mitchell 1969 Szerb et al. 1970 Aquilonius et al 1977)

Steady state levels of the precursors Ch and acetylcoenzyme A (acetyl CoA) have been less frequently analysed and only oxotremorine (OT) has been found to produce significant changes decrease in acetyl CoA and increase in Ch (Schuberth et al. 1966 Schuberth et al 1969)

The pioneer work by Quastel and co-workers demonstrated the biosynthesis of ACh in brain minces and slices and that the glucose and the K concentrations in the incubation medium are influential on synthesis, storage and release Supply of Ch did not seem to be critical in these systems (Mann et al 1939) Later MacIntosh and co-workers in the perfused superior cervical ganglion of the cat demonstrated that the supply of Ch in the perfusion medium is necessary for optimal biosynthesis of ACh during prolonged electrical stimulation of the preganglionic nerve (Birks & MacIntosh 1961)

Introduction of readily available radioactive Ch and glucose has given more detailed knowledge about biosynthesis storage and release of ACh in nervous tissue in both in vivo and in vitro preparations.

Slices from brain cortex accumulate the Ch with a carrier mediated transport mechanism, and the Ch

Table 3 *Endogenous ACh and Ch (nmol/g) in different mouse brain regions following 3 methods of sacrifice (Nordberg and Sundwall 1976)*

	Acetylcholine			Choline	
		Microwave irradiation		Microwave irradiation	
	Dislocation of the spine	Whole body 7 s	Head 0.25	Whole body 7	Head 0.25
Cerebellum	3 7±0.38 (11)	4 6±0 97 (3)	5.2±1 05 (4)	39 0±4 49 (6)	18 6±3.37 (3)*
Medulla oblongata	19 8±1.07 (12)	20.8±3 01 (3)	29 1±1 10 (4)***	44 0±7.24 (5)	46 7±11.50 (3)
Midbrain	1 1±1.24 (14)	23.–±1 47 (5)	29 4±1.65 (4)*	29.3±4 76 (7)	41.3±7.20 (3)
Striatum	37 1±2.06 (14)	40.4±3 73 (5)*	75.3±1.84 (3)****	48.8±6.50 (6)	41 4±5 65 (4)
Hippocampus	15.8±0 82 (14)	17 1±1 77 (5)	20 8±2.25 (4)	43 8±5 37 (6)	27 4±7 63 (3)
Cortex	13.2±0 74 (12)	18.4±1.55 (5)*	4 2±1.52 (4)***	30.4±2.80 (6)	30.5±3.50 (4)

In an additional sample 70 3 nmol/g
In an additional sample 48 7 nmol/g.
M ± S.E. (n) = number of experiments $P<0.05$ ** $P<0.01$ *** $P<0.001$

brain one extremely labile which can only be visualised with very short enzyme inactivation time and one rather stable about 50% of the total pool *which is not changed until after several minutes* Measurements of the radioactive ACh formed following intravenous injection of a tracer dose indicate a third pool of newly synthesized ACh with an intermediate stability (Fig. 1) It is not known how these pools relate to the free labile bound and *stable bound pools demonstrated by subcellular* fractionation

Although these experiments indicate that the

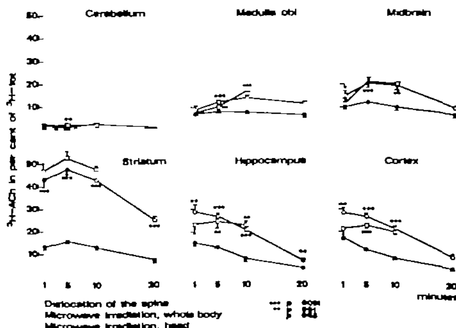


Fig 1 15 nmol of 3HCh were injected i. and the animals were sacrificed by whole body microwave irradiation, by microw ve irradiation of the head or by dislocation of the spine 1 5 10 or 20 min after injection. Each point represents the mean value of 3–5 xperiments Vertical bars indicate S. E H-tot represent the total TCA extractable radioactivity (Nordberg & Sundwall 1976)

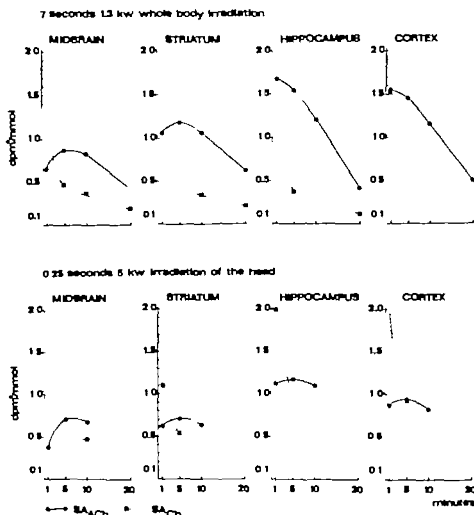


Fig 2 Time course of the specific radioactivities of Ch and ACh following an intravenous pulse injection of ³H-Ch (15 nmol/mouse) (Nordberg and Sundwall 1976)

ACh in a particular brain region is not homogenous it was found when the specific radioactivity of ACh and Ch was plotted against time a relationship which was in agreement with the direct precursor product relationship between Ch and the formed ACh (Fig.)

Calculations of turno er ha e indicated rather great regional differen the apparent turnover rate of ACh (Table 4) The highest turnover is in the striatum twice that in hippocampus and cerebral cortex which is about twi e high as in midbrain and medulla oblongata. In the cerebellum turnover is negligible

A drug like sodium pentobarb tal which earlier had been found to increase the steady state concentration of ACh in whole brain and to decrease release from the exposed cortex was found to produce a rather specific effect on the turnover of ACh in the hippocampus and cortex leaving the other brain regions unaffected (Nordberg & Sundwall 1977). OT a muscarinic agonist which also produces increased steady state levels of ACh was found to produce a marked decrease in turnover in midbrain hippocampus and cortex (Nordberg 1978)

A convulsant dose of nicotine did not change the turnover in any of the regions (Nordberg & Sundwall 1979)

Table 4 *Effect of sodium pentobarbital (60 mg × kg i p) and oxotremorine (1 mg × kg i.p) on the apparent turnover rate (nmol × g × min) of ACh in different mouse brain regions*

Brain region	Control	Sodium pento-barbital	Oxotremorine
Cerebellum	(?)	(2)	
Medulla oblongata	10	7	
Midbrain	11	9	5
Striatum	55	59	44
Hippocampus	21	6	7
Cortex	27	10	8

With the availability of radioactive Ch with extremely high specific radioactivity (60 Ci/mmol) it is possible to measure and compare turnover in extremely small regions (2 mg)

As mentioned above the sodium dependent high affinity Ch transport system appears to be located in cholinergic nerve terminals. By screening drugs known to affect turnover Kuhar and co-workers have indicated that alterations of activity in cholinergic neurons in vivo are followed by parallel changes in sodium dependent high affinity Ch uptake in vitro (Atweh et al 1975) These findings have been confirmed and extended (Nordberg 1978 Nordberg & Sundwall 1979). In fact there appears to be a linear relationship between the turnover in different brain regions and sodium dependent high affinity Ch transport measured in vitro (Fig 3) In mice pretreated with pentobarbital and OT the change in the rate of high affinity Ch uptake is related to the change in turnover However turnover appears to be a more sensitive parameter since turnover was always affected to a greater degree than high affinity uptake. When the symptoms produced by OT were reversed by atropine the effect produced by OT on turnover and high affinity Ch uptake was abolished

A convulsant dose of nicotine did not produce any effect on the high affinity Ch uptake (Nordberg & Sundwall 1979)

Even if there is yet much to be established regarding the kinetics of this high affinity uptake system for Ch the results which demonstrate a relationship between turnover and transport of Ch seem to support the view that this transport system is an important component in the regulation of the biosynthesis of ACh in the brain.

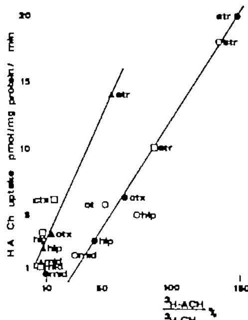


Fig 3 Relationship between ACh turnover and H.A. Ch uptake in different brain regions.
O control ▲ OT ● OT and atropine □ OT and methylatropine

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Biosynthesis and mammalian metabolism of nicotine

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ABSTRACT The biosynthesis and mammalian metabolism of nicotine is summarized

Much effort has been devoted to the elucidation of the biosynthesis of tobacco alkaloids (1) and the gathered information regard ng the biochemical precursors to nicotine [1] is presented in F g. 1

In 1960 Dawson and coworkers showed that nicotinic acid [6] is the precursor to the pyridine ring (3 4) and it was later established that the pyrrolidine ring is attached to C 3 of the nicotinic acid with loss of the carboxyl group from this position (5 6)

The precursors to the pyrrolidine ring are derived from ornithine [1] and methionine as demonstrated by the incorporation of radioactivity into nicotine when labelled ornithine was fed to excised roots or intact tobacco plants (7 8) When [? ^{14}C] ornithine was fed to *N glutinosa* or when *N tabacum* was exposed to [^{14}C ^{14}C] carbon dioxide the isolated nicotine was found to have equal labelling at C 2 and C 5 (9) indicating the formation of a symmetric intermediate [2] On feeding the [2 ^{14}C]-*N*-methyl-Δ -pyrrolinium salt [5] all radioactivity of the isolated nicotine was found in the ?'-position indicating that the randomisation of radioactivity between C 2 and C 5 in the first experiment must occur before the formation of [5]

As putrescine [2] was found to be incorporated into nicotine (10) the sequence ornithine [1] putrescine [ ] *N*-methylputrescine [3] *N*-methyl-Δ pyrrolinium salt [5] wa proposed for the biosynthesis of the pyrrolidine ring of nicotine (8). The enzymes responsible for the conversions [1–4] have later been isolated from *N tabacum* thus supporting the suggested sequence (11–13)

There has been some controversy whether this sequence via a symmetrical intermediate is major or minor route (14–16) Later results (9 17–19) support the pathway given in Fig. 1 which seems to be well established

Dawson found a much lower incorporation of radioactivity into nicotine after administration of [6-^{3}H] nicotinic acid to excised roots of *N tabacum* than after administration of the [2 ^{3}H], [4-^{3}H] or [5- H] labelled analogues (3). On feeding [6- ^{14}C H] nicotinic acid (?0) to intact tobacco plants Leete found an almost quantitative loss of tritium relative to incorporated ^{14}C. Unreacted nicotinic acid retained most of the radioactivity indicating that the loss of tritium occurred in the biological activation of nicotinic acid which is a prerequisite for its condensation with the *N*-methyl-Δ -pyrrolinium salt. The mechanism in Fig. 2 was therefore suggested for the activation of nicotinic acid and the condensation to form nicotine (21) The 3,6-dihydronicotinic acid [7] however has not been isolated Further the absolute configurations of the intermediates have not been established for the biosynthesis of nicotine (see below)

This mechanism starts with a stereospecific introduction of a hydrogen at C-6 of nicotinic acid giving [7] which via the carbanion [8] condenses with the pyrrolinium salt [5]. The product [9] is finally decarboxylated and the hydrogen originally present at C-6 is lost in a stereospecific dehydrogenation involving a hydride acceptor (Z)

In an elegant study of the stereochemistry involved in the biosynthesis of anatabine [10] (.2) (Fig. 3) both rings of which are derived from nicotinic acid (23 4), it was suggested that the activation of nicotinic acid starts with a stereospecific reduction introducing hydrogen at the pro-R position and that the final reduction in olves the stereo-

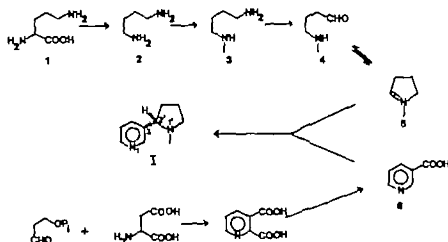


Fig 1 Biosynthesis of nicotine

specific dehydrogenation of the original pro-S-hydrogen

This should explain the complete loss of tritium from the pyridine ring and the corresponding retention of tritium in the piperidine ring.

These findings support the stereochemistry indicated in the intermediates involved in the nicotine biosynthesis

METABOLISM OF NICOTINE

The present knowledge of the mammalian metabolism of nicotine has been reviewed in detail (24–27) and the combined results from the different research groups are summarised in Schemes 1*a*–1*b* (Fig 4) (28) The major metabolite of nicotine in most species and also the first mammalian metabolite of nicotine to be isolated (29) is cotinine

The conversion of nicotine to cotinine is a two-step reaction involving two different enzyme systems and the structures IV V and VI have been postulated as intermediates (30–32)

It has been suggested that 5′-hydroxynicotine (IV) is first formed by C-oxidation involving cytochrome P-450 and that this metabolite is in equilibrium with the iminium ion V and the aminoaldehyde VI The second step is mediated by the enzyme aldehyde oxidase (30)

The iminium ion (V) has been trapped in in vitro studies (32) and a study of the position of the equilibrium between IV V and VI was recently performed (33) when the iminium ion became available through synthesis.

In a subsequent study (34) a purified aldehyde oxidase was used and it was demonstrated that the iminium ion was the most probable substrate for the enzyme The alternative substrate VI was excluded as this form could not be detected in the equilibrium mixture (n.m r) (33) Furthermore the expected product from an enzymatic oxidation of this amino-

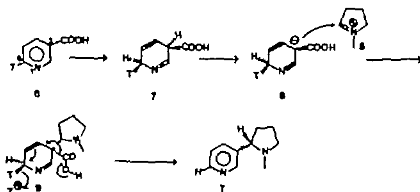


Fig 2 Activ ion of nicotinic acid and biosynthesis of nicotine

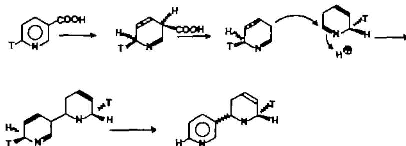


Fig 3 Biosynthesis of anatabine

aldehyde would be the amino acid VII which does not cyclize to cotinine in an enzymic process (30)

It has been suggested that a metabolite of nicotine is responsible for the established protecting effect towards normally lethal doses of nicotine that can be attained through pretreatment with sublethal doses in various species (26, 35)

The iminium ion is thus of pharmacological interest as no other metabolite so far has been shown to be responsible for this effect (26,27). As the iminium ion readily reacts with nucleophiles (31 36) it might also be responsible for the in vitro inhibition of cotinine production observed on addition of glutathione and cysteine (36) An adduct between the iminium ion and cysteine has indeed recently been observed (37)

N-Oxidation of natural S-nicotine is mediated by a microsomal flavoprotein and the two possible diastereoisomers of nicotine 1 -oxide (R, S-*cis* and S S-*trans*) are produced in varying proportions depending on the species being investigated (38 39) No further metabolism except reduction back to nicotine has been observed for these *N*-oxides, and the nicotine 1 1 -dioxide has not been isolated as a metabolite

Cotinine 1-oxide (XII) has been identified as a metabolite (40) but it has not been possible to establish whether its further metabolism occurs via X XIII or via demethylcotinine 1-oxide-XIII

Beside C and N-oxidation third type of direct metabolism has been observed for nicotine namely *N* methylation which gives nicotine isomethonium ion (II) No further metabolism of this ion into XI has been demonstrated although the latter has been isolated s a metabolite (41).

N Demethylation of nicotine and cotinine occurs in mammalian ystems although conflicting results have been published (?7) Demethylcotinine has been isolated from the urine of smokers (42) but the direct conversion X XIII has not been detected in humans given cotinine indicating that the alternative pathway I VIII XIII is prevailing (43)

In 1973 McKennis suggested that the *N*-demethylation of nicotine might proceed via a reactive methylene iminium ion ($N=CH_2$) and such an intermediate was in fact later trapped in an in vitro experiment (44). All remaining metabolites of nicotine are believed to be formed from cotinine (X) or demethylcotinine (XIII) via the common metabolite XX, or direct from nicotine via metabolites XV and XVII Hydrolysis of the lactam X (cotinine) gives the aminoacid metabolite VII (45) but no further metabolism or enzymic ring closure back to nicotine has been established for this compound (see above)

Hydroxylation of X affords 3 -hydroxycotinine (43) which is not known to give rise to any other metabolites or to 5 -hydroxycotinine (XXVI, allohydroxycotinine) The two structures XXVI and IX are believed to be in equilibrium (?6 46) and both ha e been isolated as metabolites of nicotine (?6 43 47 48) However H and ^{13}C n.m.r data have shown that only the cyclic isomer is present at physiological pH (26, 44 49)

5 -hydroxycotinine or its open form IX is metabolized further to the ketoacid XX (50) and the corresponding hydroxyacid (XXII) (51) via oxidative deamination and reduction of the ketone In slightly acidic solutions the latter metabolite gives rise to the lactone XXIV spontaneously (26) and although this isomer has not been isolated as a nicotine metabolite it furnishes the remaining metabolites XVII and XXVII when fed to rats (26)

3 pyridylacetic acid (XXVII) is considered to be the ultimate metabolite of nicotine (5? 53), but its conjugate with glycine (XXIX) has been isolated (26) No evidence for the conversion of XXVII to nicotinic acid has been found.

Scheme 1a Abridged scheme for the mammalian metabolism of nicotine

Scheme 1b Abridged scheme showing ions and N-oxides implied in the mammalian metabolism of nicotine

Fig 4

Dihydrometanicotine (XV) presumably formed from nicotine by way of benzylic oxidation and ringopening to *N*-methyl-4-(3-pyridyl)-3-butenylamine (metanicotine) has been suggested as another source of the ultimate metabolite XXVII as both this acid and XVII could be isolated from urine of dogs to which XV had been administered (54)

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Studies of muscarinic and nicotinic binding sites in brain

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ABSTRACT B nding properties for the muscarinic antagonist quinuclidinyl benzilate (QNB) the nicotinic antagonist tubocurarine and nicotine to crude synaptosomal fractions from mouse and rat brain have been studied Marked regional differences in number of binding sites for labelled QNB (^{3}H-QNB) were found with high concentration in the striatum cortex and a low concentration in the cerebellum. The specific binding sites for labelled tubocurarine (^{3}H-tubocurarine) showed less regional variation An increased number of muscarinic binding sites (with no change in receptor affinity) was found in rats following long-term treatment w th barbital

The introduction of high affinity ligands of high specific radioactivities has accelerated the receptor binding studies in brain during recent years. For studies of the muscarinic receptor several antagonists (atropine propylbenzylcholine methyl scopolamine quinuclidinyl benzilate) and agonists (oxotremorme-M pilocarpine) have been used as ligands (Yamamura & Snyder 1974 Burgen & Hiley 1975 Birdsall et al 1978 Hulme et al 1978 and Kloog & Sokolovsky 1978) Concerning the nicotinic receptor most studies have been performed in electric eel and peripheral nervous tissue and very little in brain. α-Bungarotoxin a neurotoxin which is known to act as an irreversible antagonist of nicotinic receptors in electric fish and skeletal muscle has recently also been used for nicotinic receptor binding studies in brain. The evidence for and against α-bungarotoxin as a nicotinic receptor ligand however has recently been discussed by Schmidt (1977) and Morley et al (1979)

In the present study we have measured both muscarinic and nicotinic binding sites in brain. For the studies of muscarinic binding sites the antagonist quinuclidinyl benzilate (QNB) was used as radiolabelled ligand and for the nicotinic receptor binding studies the ntagonist tubocurarine and nicotine tself were used The regional distribution of muscarinic and nicotinic binding sites in brain are compared I addition some data obtained in col laboration with Dr G Wahlström are presented indicating an increased number of muscarinic binding sites in brain in the abstinence following long-term forced oral barbital administration.

MATERIALS AND METHODS

Binding assays A crude ynaptosomal fraction (P frac tion) (Gray & Whittaker 196.) was prepared from different parts of mouse and rat brain. The P fraction was diluted to protein concentration of 1–4 mg/ml 100 μl of the P fraction were added to 900 μl N KPO buffer (pH 7.4 containing tritium labelled ligand) and incubated for 2–60 m at 0–25°C The incubation was terminated by cooling the samples t 0°C in ice followed by centrifugation in microfuge® (Beckman) for 5 min The tip of the tubes were cut and the pellet dissolved in soluene/toluene (1 3) over night (Terenius & Wahlström 1975) 14 ml scintillation mixture containing toluene methanol and Permablend were added and the radioactivity wa measured by liquid scintillation. Specific binding of ^{3}H-QNB ^{3}H-tubocurarine and ^{3}H-nicotine were calculated by subtracting the value for nonspecific binding in presence of 100 μM oxotremorine (+) tubocurarine or nicotine from the total binding in absence of unlabelled ligand The protein content of the P fraction was measured (Lowry et al. 1951) and bovine albumine was used as standard.

Radiolabelled ligand [13 ^{3}H] Tubocurarine chloride dextro (16.48 Ci/mmol) was purchased from New England Nuclear

[G-^{3}H]Nicotine d-bitartrate (3 Ci/mmol) was purchased from the Radiochemical Center Amersham.

[3-^{3}H] Quinuclidinyl benzilate (16 Ci/mmol) was purchased from the Radiochemical Center Amersham

Chronic barbital treatment Male Sprague-Dawley rat were chronically receiving sodium barbital (3.33 mg/ml) in

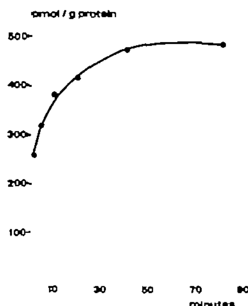


Fig. 1 Specific binding of H-QNB as function of time of incubation 0.4 nM ³H-QNB (racemate) were incubated with crude synaptosomal (P) fractions from the mouse cortex for different times (2–80 min). Each point represents the mean value of 3 experiments. ● specific binding; ▲ unspecific binding.

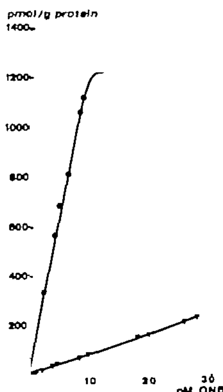


Fig. 2 Specific binding of H-QNB as function of H-QNB concentration. Different concentrations of H-QNB were incubated with P² fractions from the mouse cortex at 25°C for 60 min. Each point represents the mean value of 3 experiments ● specific binding; ▲ unspecific binding.

their drinking fluid The daily consumption of barbital was about 200 mg/kg/day After about 40 weeks of treatment the barbital solution was replaced by water One group of animal were killed at the time of withdrawal of the barbital solution and tw other groups of animal were killed after 3 and 12 days of abstinence Abstinence convulsions were recorded For further detail regarding the chronic treatment see Wahlstrom (1974) and Nordberg et al. (1979)

RESULTS AND DISCUSSION

Fig. 1 show that the specific binding of H-QNB to a crude synaptosomal fraction (P fraction) is maximal after 40 min of incubation at 25°C The specific binding i about 90% of the total binding. Incubation with different concentrations of H QNB indicates a saturation of the binding at about 1 nM H-QNB (Fig) Muscarinic agonists and antagonists can displace H-QNB from its binding sites. Inhibition curves for atropine and oxotremorine are shown in Fig 3 IC_{50} values of 5 × 10 M for atropine and 7 10 M for oxotremorine are obtained (Fig. 3) Thus hundred time difference in IC_{50} value is found between the agonist and antagonist

The distribution of H-QNB binding sites in brain is shown in Table I The highest content of binding

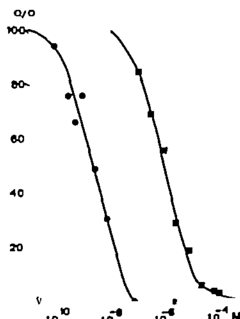


Fig. 3 Inhibition by tropine (●) and oxotremorine (■) of the specific binding of ³H-QNB (0.4 nM racemate) to P² fraction from the mouse corte Each point represent the mean value of 3 periment

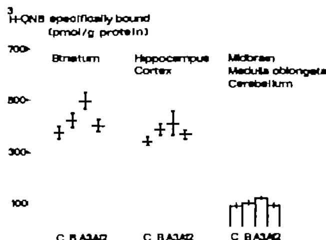


Fig 4 Effect of long term barbital treatment on number of ^{3}H-QNB binding sites i different regions of rat brain. C=control B=barbital until sacrifice A3=abstinent for 3 days, A12=abstinent for 12 days. Each column represents the mean value from 6–9 animals (Nordberg et al. (1979)).

sites is found in the cortex striatum intermediate in the hippocampus midbrain medulla oblongata and lowest in the cerebellum. About the same number of binding sites are found in the striatum and cortex although the content of endogenous acetylcholine (ACh) ACh turnover and high affinity synaptosomal choline (Ch) uptake are 2–3 times higher in the striatum in comparison with the cortex (Nordberg 1977 1978) Thus there seems to be no obvious relationship between ACh turnover and number of muscarinic binding sites

Chronic treatment with barbital can increase the number of muscarinic binding sites in brain (Nordberg et al 1979) Fig. 4 shows the results from an experiment where rats had been treated for 40 weeks. When the barbital solution was withdrawn (*B*) no significant effect on the number of ^{3}H-QNB binding sites was found. 3 days later (A3) when abstinence convulsions were maximal a 30% higher number of ^{3}H-QNB binding sites was found in the striatum and midbrain preparations. On the 12th day of abstinence the number of binding sites had returned to control level A Scatchard plot of the binding data for the midbrain preparation showed an increase in B_{max} with 50% on the 3rd day of abstinence in comparison with control No difference was found in the receptor affinity Other studies indicate an increased turnover of ACh during the early part of the barbital abstinence (Nordberg & Wahlstrom 1977 1979) This is as far as we know the first study showing that chronic drug treatment can increase the number of muscarinic binding sites in brain

Table 1 *Regional distribution of ^{3}H-QNB a d H tubocurarine binding sites in brain*

Crude synaptosomes from mouse brain were incubated with ^{3}H-QNB (0 4 nM racemate) for 60 min at 25°C Crude synaptosomes from rat brain were incubated with ^{3}H tubocurarine (3 nM) for 15 min t 25°C Mean values of 2–5 experiments

Brain region	^{3}H-QNB binding (pmol/g protein)	^{3}H-tubocurarine binding (pmol/g protein)
Cortex	561	144
Striatum	56_	104
Hippocampus	471	99
Midbrain	320	1__
Hypothalamus		135
Medulla oblongata	222	140
Cerebellum	94	99

Table 2. *IC_{50} values for t bocurarin and nicotine competing th H-tubocurarine (3 nM) and H nicotine (70 nM)*

	IC_{50} 10^{-8} M	
Labelled ligand	Tubo-curarine	Nicotine
^{3}H-tubocurarine	2	65
H-nicotine	50	100



Fig 5 Inhibition by tubocurarine of the specific bindi g of H-tubocurarine (3nM) to P2 fractions from the mouse hippocampus The incubation was performed t 25°C for 15 min Each point represents the mean value of 3 experiments.

The binding of ³H-nicotine and ³H-tubocurarine to a P fraction from the hippocampus can be measured at 0°C The ³H-nicotine binding is the same at 0°C and 25°C while the H-tubocurarine binding increases with 50 % in the temperature range 0°C to 25°C. The specific binding for both ligands is about 30–40 % of the total binding. A maximal specific binding is obtained after about 5 min of incubation

The ³H-nicotine and ³H tubocurarine binding can be blocked by increasing concentrations of unlabelled ligands. Table 2 shows IC_{50} values for nicotine and tubocurarine obtained after incubation with H-nicotine and H-tubocurarine As can be seen in Table tubocurarine is 25 times more potent in inhibiting H-tubocurarine binding in comparison with ³H-nicotine binding For nicotine about the same IC_{50} value is obtained with both labelled ligands

The specific binding of H-tubocurarine to crude synaptosomes increases linearly with increasing concentrations up to 10 nM (concentration range 1–10 nM) A Scatchard plot of the binding data indicates two different binding sites with a K of 1.5 and 14 nM respectively Binding studies in brain using labelled α-bungarot xin or naja-naja siamen sis toxin have shown ne binding te Etorovic & Bennett (1974) found a f 3 nM us ng α-bungarotoxin and Speth et al. (19) A of 0 7 nM with naja-naja toxin The pos ib l t of more than one bungarotoxin binding sit ha been dis sed by Morley et al (1979) Fig 5 show the h bition of H tubocurarine binding w th unlabel ed tubocurarine at several different ncentration t un labelled tubocurarine As can be seen in Fig the inhibition curve is very flat and has a shape which might indicate more than one binding site

The distribution of ³H-tubocurarine binding sites in different part of the brain is at present under investigation Some recently obtained data are given in Table 1 and as can be concluded from the table the ³H tubocurarine binding sites are more even distributed in brain in comparison with the ³H-QNB binding sites (Table 1)

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Effects of neonatal nicotine administration on the postnatal development of central noradrenaline neurons

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ABSTRACT The effects of neonatal nicotine and/or 6-hydroxydopamine (*6-OH-DA*) treatment on the postnatal development of central noradrenaline (NA) neurons have been investigated using neurochemical and histochemical techniques. Nicotine was found to produce an increase of ^{3}H-NA uptake and endogenous NA in the cerebral cortex and pons-medulla, which was most pronounced at the age of one week. These parameters were normalized in the adult stage. Neonatal nicotine treatment was also found to partially counteract the 6-OH DA induced alteration of the development of the *locus coeruleus* NA system. The NA denervation produced by 6-OH-DA in the cerebral cortex and the spinal cord was thus counteracted by nicotine treatment. The results suggest that neonatal nicotine administration has a growth stimulatory effect on the early postnatal development of central NA neurons

The central catecholamine (CA) neurons are known to appear early in gestation in many species including man. The CA perikarya are developed at birth while their nerve terminal projections continue to develop postnatally and reach adult nerve density about one month after birth (see Jonsson 1976). Previous studies have shown that the postnatal development of the central noradrenaline (NA) neurons is normally highly ordered and strictly programmed from a structural standpoint indicating a high degree *of intrinsic growth regulation.* However very little is known as to mechanisms involved in governing growth processes and the development of the regional innervation pattern of central NA neurons. In view of the findings that the preganglionic cholinergic input is of great importance for the development of the end organ innervation by the postganglionic sympathetic neurons in the peripheral nervous system (Black & Mytilineou 1976) *it was considered of interest to investigate* whether or not nicotine can affect the postnatal development of central NA neurons.

MATERIALS AND METHODS

Albino rats (Sprague-Dawley) were used. The drug treatments were started on the day of birth and four groups of animal were studied A *Controls* B *Nicotine* (3–4×2 mg/kg s.c 24 h intervals C. *6-OH DA* (100 mg/kg s.c., within 4 h after birth); D *6-OH-DA* (100 mg/kg s.c.) *and nicotine* (3–4 mg/kg s.c., 24 h intervals. The first nicotine injection was given 3–4 h after the 6-OH-DA administration). The animals were killed by decapitation. ^{3}H-NA uptake (0.05 μM 5 min) was measured in vitro in homogenates according to Jonsson et al. (1974). Endogenous CA concentrations were determined by using high pressure liquid chromatography with electrochemical detection (Keller et al 1976). The fluorescence histochemical method of Falck Hillarp for the demonstration of biogenic monoamines was utilized to study the fluorescence morphology of the CA neurons (Falck et al 1962, Corrodi & Jonsson 1967).

RESULTS AND DISCUSSION

Neonatal nicotine treatment was found to produce a 15–30% increase in ^{3}H NA uptake in vitro and endogenous NA levels in the neocortex one week after the treatment while there was a normalization *of these parameters in the adult stage. Similar ef*fects were observed in the pons-medulla although less pronounced. These data indicate that nicotine has a growth stimulatory effect on the NA nerve terminals although not persistent which may be due to the short period of nicotine administration. Analysis of the effects of neonatal nicotine and/or 6-OH-DA treatment on the regional CA concentrations in the adult stage showed that nicotine treat-

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Mecamylamine induced blockade of nicotine induced inhibition of gonadotrophin and TSH secretion and of nicotine induced increases of catecholamine turnover in the rat hypothalamus

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ABSTRACT Nicotine treatment of normal male rats and of ovariectomized female rats results in increases of dopamine (DA) noradrenaline (NA) and adrenaline (A) turnover in the hypothalamus especially within the median eminence. These effects may in part mediate the inhibition of gonadotrophin and TSH secretion produced by nicotine.

Previous work has indicated that in the normal male rat and in the ovariectomized female rat, nicotine may reduce LH and prolactin secretion via an activation of the tubero-infundibular dopamine (DA) neurons to the lateral and medial palisade zone of the median eminence respectively (Fuxe et al 1977 Eneroth et al 1977) In order to evaluate if these changes are related to the activation by nicotine of central nicotine-like cholinergic receptors it has in the present study been analysed if the ganglion blocking agent mecamylamine can block the hormonal effects and catecholamine (CA) turnover effects of nicotine within the hypothalamus.

MATERIALS AND METHODS

Normal male specific pathogen free Sprague-Dawley rats and 1 month and 3 month ovariectomized female specific pathogen free Sprague-Dawley rats have been used Nicotine was injected alone or together with mecamylamine the dose and time schedule shown in legend to Table 1 3 A possible change in CA turnover was evaluated by studying depletion of the CA stores following treatment with the tyrosine hydroxylase inhibitor α-methyl-tyrosine methylester (H 44/68 250 mg/kg p 1 or h before killing) (Andén et al 1969). In the regional analyses of CA stores in the various parts of the hypothalamus Falck-Hillarp technique for the demonstration of the cellular localization of CA was used in combination with quantitative microfluorimetry (see Fuxe et al 1977). In the biochemical analysis of the CA stores in the whole rat hypothalamus high pressure liquid chromatography was used in combination with electrochemical detection (Keller et al 1976) Determinations of serum levels of LH FSH prolactin and TSH were obtained by means of radioimmunoassay using kits for the assay of LH FSH prolactin and TSH which were kindly supplied by The National Institute for Arthritis Metabolism and Digestive Diseases (NIAMDD) rat pituitary hormone distribution program (see Fuxe et al. 1977 Eneroth et al 1977).

RESULTS

As seen in Table 1 nicotine treatment in repeated doses of mg/kg produces a significant depletion of the CA stores in the various parts of the median eminence of the ovariectomized female rat. The experiment with H 44/68 revealed an enhancement by nicotine of the H 44/68 induced depletion of CA stores in the various parts of the median eminence In the forebrain, on the other hand nicotine could not deplete the DA stores in tuberculum olfactorium, nuc accumbens and nuc. caudatus In the nuc accumbens nicotine reduced the H 44/68 induced depletion of the DA stores while nicotine did not influence the other two regions The results obtained on hormone levels in ovariectomized female rat are summarized in Table Nicotine in the same dose range as above produced marked inhibition of prolactin LH FSH and TSH serum

Table 1 *Effect of nicotine on CA fluorescence and H 44/68 induced CA fluorescence disappearance in various regions of the 3 month ovariectomized female rat*

Nicotine (2 mg/kg, i p) was given 4 times with 30 min intervals, the animals being killed 30 min after the last injection. H 44/68 (250 mg/kg, i.p) was given 2 h before decapitation in the saline treated group and 5 min after the first injection of nicotine in the nicotine treated group Means ± S E in per cent of saline treated group means Number of animals within parenthesis In the statistical analysis Mann–Whitney U-test was used. $P<0.05$ $P<0.01$ $P<0.001$ SEL subependymal layer of the median eminence· MPZ = medial palisade zone of the median eminence LPZ = lateral palisade zone of the median eminence TO = tuberculum olfactorium ACC = nucleus accumbens CAUD = nucleus caudatus

Treatment	Dose (mg/kg)	SEL	MPZ	LPZ	TO	ACC	CAUD
Saline		100±5 (7)	** 100±3 (7)	100±6 (7)	100±5 (7)	100±7 (7)	100±8 (7)
Nicotine	4×2	64±4 (7)	80±4 (7)	84±4 (7)	92±7 (7)	95±6 (7)	104±9 (7)
Saline + H 44/68		69±6 (7)	43±4 (7)	43±2 (7)	63±6 (5)	57±3 (7)	72±3 (7)
Nicotine + H 44/68	4×2	43±3 (7)	33±3 (7)	32±2 (7)	60±6 (7)	68±4 (7)	77±4 (7)

Table 2. *Effect of mecamylamine on the nicotine + H 44/68 induced changes in PRL LH FSH and TSH levels of the 1 month ovariectomized female rat*

Ovariectomy was performed 1 month before killing. Nicotine (? mg/kg, i.p) was given immediately before H 44/68 (250 mg/kg, i.p h before killing) and 30 60 and 90 min after H 44/68 Mecamylamine (1 mg/kg, i.p) was given 1 h before and 1 h after H 44/68 Means ± S.E of hormone levels are given in per cent of saline + H 44/68 treated group means. Number of animals in parenthesis. Statistical analysis according to Wilcoxon, one-way classification comparing all possible pairs of treatments $P<0.05$ ** $P<0.01$ In the saline + H 44/68 treated group the serum levels of TSH ere 1223±138 ng/ml the serum prolactin levels were 265±37 ng/ml the serum LH levels were 469±3 ng/ml and the serum FSH levels were 2 197±64 ng/ml

Treatment	Dose (mg/kg)	PRL (%)	LH (%)	FSH (%)	TSH (%)
Saline + H 44/68		100±14 (7)	100± 7 (7)	100±3 (7) **	100±11 (7)
Nicotine + H 44/68	4×2	14± 1 (5)	43± 7 (5)	79±3 (5)	43± 6 (5) **
Mecamylamine + ine + H 44/68	2×1 4 2	35± 7 (6)	65± 6 (6)	92±6 (6)	77±10 (6)
ecamylamine + H 44/68	2×1	100± 5 (7)	91±10 (7)	88±6 (7)	157±13 (7)

Table 3 *Reduction by mecamylamine of nicotine induced increases in the H 44/68 induced depletion of NA A and DA stores in the hypothalamus of th normal mal rat*

Nicotine (2 mg/kg s.c.) was given 2 times with 30 min intervals, the first dose given immediately before H 44/68 (250 mg/kg i.p 1 h before decapitation) Mecamylamine (1 mg/kg c) was given immediately before the first nicotine or saline injection Means ± S.E. in per cent of H 44/68 treated group means ~=6. Statistical analysis according to Wilcoxon: one-way classification, comparing all possible pairs of treatments. $P<0.05$ $P<0.01$ NA 100%= 841±162 ng/g tissue A 100%=76±5 ng/g tissue DA 100%=263±23 ng/g tissue.

Treatment	NA (%)	A (%)	DA (%)
Saline + H 44/68	100± 6	100±6 **	100± 9
Nicotine 2×2 mg/kg + H 44/68	38± 3	41±1	59± 5
Mecamylamine 1 mg/kg + nicotine 2 mg/kg + H 44/68	87±10	86±5 **	103±12
Mecamylamine 1 mg/kg + H 44/68	86±10	103±8	76±10

levels in the presence of the tyrosine hydroxylase inhibitor Mecamylamine treatment with two doses of 1 mg/kg partly seemed to counteract the nicotine induced inhibition of gonadotrophin and TSH secretion Mecamylamine alone did not by itself produce any significant changes in hormone secretion but there was a trend for an increase in TSH secretion following mecamylamine treatment in combination with tyrosine hydroxylase inhibition The results obtained in the biochemical analysis of the CA stores in the whole rat hypothalamus following treatment with nicotine are shown in Table 3 It is seen that nicotine produces an enhancement of the H 44/68 induced depletion of the noradrenaline (NA) adrenaline (A) and DA stores in the normal male rat hypothalamus The enhancement of the H 44/68 induced depletion of all the CA stores is blocked by pretreatment with mecamylamine in a dose of 1 mg/kg. Mecamylamine in doses of 3–10 mg/kg by itself induced an enhancement of the H 44/68 induced depletion of the DA and A stores in the hypothalamus A similar but not significant, effect was present within the NA systems.

DISCUSSION

The present findings give further evidence that nicotine treatment can reduce the DA and NA stores within the median eminence and that it increases DA and NA turnover within this region Mecamylamine pretreatment, as shown in the biochemical experiment resulted in a blockade of the nicotine induced increases of NA and DA turnover found within the hypothalamus Thus evidence is given that the nicotine induced increases of DA and NA turnover are due to the activation of cholinergic nicotine-like receptor probably located within the hypothalamus The discovery was also made that nicotine can increase A turnover within the hypothalamu an effect which also is blocked by mecamylamine pretreatment Thus nicotine-like cholinergic receptors may also control the release of A from adrenergic terminals within the hypothalamus Mecamylamine pretreatment could also in part counteract the nicotine induced inhibition of gonadotrophin and TSH secretion It therefore seems possible that also the hormone changes induced by nicotine are related in part to stimulation of a cholinergic nicotine-like receptor It is suggested that cholinergic nicotine-like receptors exist on the DA NA and A terminals within the hypothalamus and that their activation by nicotine can result in inhibition of gonadotrophin and TSH secretion at least in the dose range used in the present analysis.

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Effect of nicotine on single cell activity in the noradrenergic nucleus locus coeruleus

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ABSTRACT The effect of nicotine and various cholinergic and anticholinergic drugs on brain noradrenaline (NA) containing neurons in the locus coeruleus (LC) of the rat was studied utilizing single cell recording techniques and microiontophoretic drug application. Nicotine (10–90 μg/kg i v) caused a dose-dependent, short-lasting increase in the firing rate of NA neurons in the LC. When microiontophoretically applied nicotine had no effect on LC neurons, which in contrast were activated by acetylcholine (ACh) and the muscarinic agonist, bethanechol The stimulation of NA neurons in the LC by i. physostigmine was antagonized by scopolamine but not by methylscopolamine. Also microiontophoretically applied scopolamine specifically antagonized the NA-cell activation by ACh. Consequently nicotine probably causes an indirect activation of NA neurons in the LC. These cells seem to be equipped with excitatory cholinergic receptors of muscarinic but not nicotinic character The LC activating effect of nicotine may be important for its arousal producing properties.

Key words Nicotine, NA neurons, Locus coeruleus muscarinic receptors, arousal

Previous experiments have shown that nicotine causes an increased release of ^{3}H noradrenaline (^{3}H NA) into the effluent from the perfused cerebral ventricle of the cat (Hall and Turner 1972) The drug also increased the release of ^{3}H NA from slices of rat hypothalamus and cerebellum (Westfall 1974). Interestingly also physostigmine like oxotremorine seemed to activate brain NA mechanisms since it caused acceleration of the α-methyl-p-tyrosine induced disappearance of brain catecholamines (CA) (Corrodi et al 1967) as well as increased the central CA synthesis rate (Andén & Wachtel 1977) Recently the NA neurons in the locus coeruleus (LC) which innervates almost the entire brain and spinal cord (Ungerstedt 1971 Lindvall & Bjorklund 1974 Nygren & Olsson 1977) were found to be excited by microiontophoretically applied acetylcholine (ACh) (Bird & Kuhar 1977) Thus, the present study was undertaken to characterize the effect of nicotine on cholinergic receptor within the LC by utilizing single cell recording techniques and microiontophoretic application of various cholinergic agonists and antagonists onto LC noradrenergic neurons

METHODS

Male Sprague-Dawley rats (230–280 g) were used. The electrophysiological experiments were performed essentially as has been described previously (Svensson et al 1975 Svensson & Usdin 1978). The animals were anesthetized with chloral hydrate (400 mg/kg i p) and mounted in stereotaxic apparatus. Additional injections were given as needed. Although general anesthetics seem to block excitatory muscarinic actions of ACh on cortical neurons (Catchlove et al 1972) this antagonism is not seen after chloralhydrate e.g on hippocampal pyramidal cells (Bird & Aghajanian 1975) and also in the present study excitatory muscarinic effects of ACh were readily obtained A 3 mm burr hole was drilled with its centre located 1 1 mm lateral to the midline and 1 1 mm posterior to lambda For single-barrel experiments a micropipette with tip diameter of 1 μm filled with M NaCl saturated with fast-green (impedance in vitro 3–7 MΩ measured at 135 Hz) was lowered with hydraulic microdrive into the brain. The electrode potentials were passed through high input-impedance amplifier and filters. Each spike was discriminated and fed into an integrator being reset every 10 or 1 and finally displayed on an oscilloscope an audiomonitor and an oscillographic recorder Drugs were administered into tail vein. The body temperature of the animals was kept at 36–37°C Only one cell was recorded in each animal. In some experiments microiontophoretic techniques were used utilizing circuitry essentially the same as previously described (Salmoiraghi & Weight 1967) except that solid state design was chosen Micropipettes with tip diameter of 4–5 μm were used with few strand of fiberglass inside prior to pulling to facilitate direct filling of the tips by capillary action (Tasaki et al 1968). The central barrel was filled with 2 M CaCl solution saturated with fast green as above and as used for recording action potentials One of the side barrel contained 4 M CaCl solution for automatic current balancing and the ot

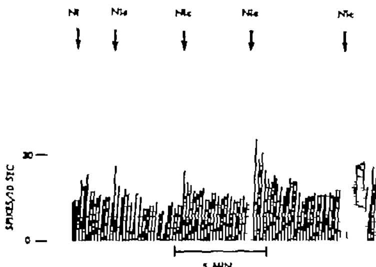


Fig 1 Typical record showing the stimulation by nicotine (Nic 10+20+20+50+50 µg/kg i administered) of the firing rate of a noradrenergic neuron in the locus coeruleus in the rat brain. The effect is almost instantaneous of short duration and dose-dependent.

contained drug solutions as listed below The drug barrels contained 0 05 M or 0.2 M acetylcholine chloride pH 6.2 (Sigma) 0 02 M, 0 1 M or 0.2 M nicotine bitartrate, pH 4 0 0.2 M betanechol chloride, pH 5.5 (Sigma); 0 02 M L-glutamic acid in 0.2 M NaCl pH 8 6 (B.D H) and/or 0.1 M scopolamine hydrochloride pH 4 7 (ICN) The impedances were typically in vitro 2.5–5 MΩ in the central barrel 20–100 MΩ in drug barrels. A retaining current of 10 nA was maintained between ejections, which were performed frequently to void dead-time artefacts.

A tentative LC neuron was localized utilizing several criteria (Korf et al 1974) including its localization just ...al to the large cells of the mesencephalic nucleus of the Vth nerve which typically respond to gentle movement of the jaw by bursts of activity Furthermore the LC cell are located just below the 4th ventricle a zone of electrical silence and the spikes have a typical positive-negative wave form often with notch on the ascending limb The characteristic rhythm and frequency (1–5 imp/s) and the typical esponse to noxious stimuli by a burst in activity followed by quiescent interval (for details see Cedarbaum & Aghajanian 1978) was also observed The final recording site was marked at the end of each experiment by iontophoretic ejection of fast-green The rats were then perfused through the heart with 10% formaldehyde and serial 50 µm frozen section of the brain were cut, mounted and stained with cresyl iolet and counterstained with neutral red Only units within the LC were included in this study

RESULTS

When administered i.v in small doses (10–50 µg/kg) nicotine caused a dose-dependent shortlasting increase in the firing rate of the NA neurons in the LC (Fig. 1) Also physostigmine consistently produced increased firing of the LC-neurons and this effect was obtained even after pretreatment with methylscopolamine In contrast scopolamine rapidly antagonized the physostigmine induced LC activation (Fig. 2) The microiontophoretic experiments revealed that nicotine, in contrast to ACh and the muscarinic agonist, bethanechol (Fig 3) did not activate the LC-cells, when tested in the various concentrations and at different ejection currents (0–50 nA) These experiments also showed that the muscarinic antagonist, scopolamine totally blocked the ACh-induced excitation of LC neurons This blocking effect was obtained in a dose that did not at all inhibit the glutamate induced excitation of these cells (Fig. 4) Other presumed nicotinic antagonists [(+)-tubocurarine hexamethonium or gallamine] did not block the ACh excitation (data not shown)

DISCUSSION

The present experiments show that nicotine in relatively small doses (10–50 µg/kg i v) causes a dose dependent shortlasting activation of NA-neurons in the LC The short duration is in accordance with the very rapid accumulation in and disappearance from brain tissue previously reported (Schmiterlöw & Hansson 1965) The doses of nicotine

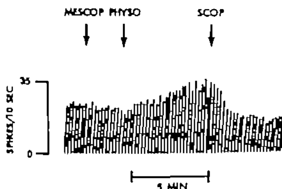


Fig 2 Pretreatment with the peripherally but not centrally active muscarinic receptor blocking agent methylscopolamine (MESCOP 1 mg/kg i.v.) did not prevent the stimulation of the impulse activity of NA neuron in the LC by threshold dose of physostigmine (PHYSO 40 µg/kg i.v.). This effect was, however readily antagonized by the centrally active antagonist scopolamine (SCOP 0.25 mg/kg i.)

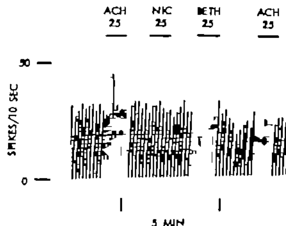


Fig 3 Integrated rate histogram showing the stimulation by microiontophoretically applied acetylcholine (ACh) and bethanechol (BETH) of the firing rate of a NA-neuron in the LC. In contrast, nicotine (NIC) did not cause any excitation. The drugs were applied at 25 nA for 1 min ejection periods (horizontal bars)

equivalent to those which produce cortical activation and behavioural arousal in animals (see Hall 1970) Recent studies have implicated the LC in maintenance of wakefulness and arousal reactions (Fuxe et al 1970 Foot & Bloom 1978 see Redmond 1977) as well as in modulation of autonomic e.g. cardiovascular function (see Amaral & Sinnamon 1977) Thus, electrical stimulation of the LC produced increased apprehensiveness unrest and anxiety reactions in monkeys (Redmond et al 1976) as well as a pressor response in rats and cats (Przuntek & Philippu 1973 Ward & Gunn 1976) Consequently the stimulation by nicotine of LC neurons may form part of the underlying mechanism for such reactions in man following nicotine administration although the hypertensive response probably mainly is related to facilitated ganglionic transmission (Gebber 1969) The duration of the stimulatory effect of nicotine on brain NA-neurons corresponds to that of the hypertensive response obtained by the same doses (Armitage et al 1968 Matsuoka & Domino 1977). The LC stimulation is however probably not secondary to this response since acutely increased blood pressure e.g. obtained by intravenous injection of NA is not accompanied by increased LC cell firing rate (Engberg & Svensson unpublished observations)

The present data clearly suggest that the nicotine-induced LC stimulation is not directly mediated by cholinergic nicotinic receptors on the NA-neurons. This is so since both the LC stimulation by physostigmine and that of ACh when microiontophoretically applied, was blocked by scopolamine but not by the presumed nicotinic antagonists The lack of antagonism by methylscopolamine indicates that peripheral muscarinic effects of physostigmine are of little significance for its LC stimulating action. Furthermore the muscarinic agonist bethanechol caused activation

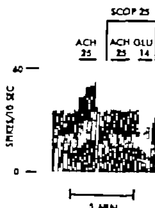


Fig 4 The ACh-induced increase in firing rate of NA cells in the LC totally blocked by the simultaneous microiontophoretic application of scopolamine (SCOP 25 nA) However the probably non-specific activation of the NA neuron by application of glutamate (GLU 14 nA) was unaffected by the muscarinic antagonist.

of LC neurons in contrast to nicotine when microiontophoretically applied Thus the cholinergic excitatory receptor in the LC is of muscarinic character This view is supported by the specificity of the ACh antagonistic action of scopolamine which did not block the NA-cell excitation by glutamate — a noncholinergic generally excitant amino acid (see Krnjević 1974)

In conclusion nicotine in small doses causes a probably indirect activation of NA neurons in the LC which are equipped with excitatory muscarinic cholinergic receptors

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Effect of chronically administered nicotine on axonal transport of dopamine-β-hydroxylase in peripheral adrenergic neurons and on blood pressure and heart rate in the rat

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ABSTRACT Nicotine was given to rats of about 200 g b wt. in the drinking water for up to 2 months. Different doses of nicotine, from 25 mg to 100 mg per litre water were tested with respect to their reducing effect on the normal increase in body weight. No effect was found on the normal accumulation of dopamine-β-hydroxylase (DBH) proximal to ligation of peripheral adrenergic neurons This indicated normal turnover of amine storage granules. This was interpreted as nicotine being without effects on the activity of the adrenergic neurons In agreement with this lack of effect no changes of blood pressure or heart rate were found in rats receiving 50 mg nicotine per litre of drinking water

Key words Nicotine chronic treatment, sympathetic activity dopamine-β-hydroxylase transport, blood pressure heart rate

Effects of nicotine on the peripheral autonomic system have been studied for more than one hundred years (for review see Trendelenburg 1965) The pharmacological properties of nicotine are very complex with stimulating as well as inhibiting actions on both the peripheral nervous system and the central nervous system. Much attention has been paid to the stimulating effect of nicotine on the sympathetic ganglia and the adrenal medulla. A directly stimulating effect of nicotine on the peripheral adrenergic nerve terminals has also been reported (e.g. Su & Bevan 1970 Westfall & Brasted 1972, Nedergaard & Schrold 1977) Nicotine may thus by acting at different levels in the adrenergic neuron increase the activity of the neuron and cause increased release of the transmitter noradrenaline (NA)

Such an increased sympathetic activity has been discussed to cause stimulation of the cardiovascular system giving increased blood pressure and increased heart rate (see e.g. Volle & Koelle 1975). In most experiments, however nicotine has been given in a single dose, while smoker is exposed to chronic treatment with nicotine.

Studies on the long-term effect of nicotine on the activity of the adrenergic neurons and on the cardiovascular system are rather few and the results are partly contradictory (compare e g. the results of Westfall 1970 with those of Wenzel & Azmet 1970)

The activity in sympathetic adrenergic neurons has been studied indirectly by measuring the amount of dopamine-β-hydroxylase (DBH) accumulated per time unit above a crush or a constriction of adrenergic axons. The background for this approach was as follows. The transmitter NA is stored in amine storage granules (Euler & Hillarp 1956) The NA has to be stored in granules in order to be released from the nerve terminals during physiological nerve activity (cf Andén Carlsson & Häggendal 1969) The granules are complex structures containing not only NA but also proteins e g. DBH and during nerve activity NA and also to some extent DBH will be released (cf Geffen & Livett 1971) The granules will thus be worn-out and have to be replaced by newly formed granules. In all probability the new granules are formed in the cell bodies and transported down to the nerve terminals (cf Dahlström 1971 Häggendal & Dahlström 1971) The amounts of granular components, such as DBH or NA, accumulating above a constriction of the

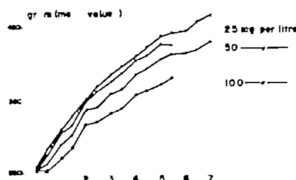


Fig 1 Body weights of rats with or without nicotine in the drinking water The values indicated are mean values calculated from groups of 5 animals. 2–5 groups have been used for every point. Nicotine was given as bitartrate and the doses indicated correspond to pure nicotine

axons will reflect the production of new granules This production is increased at increased nerve activity and decreased at decreased nerve activity in agreement with "trans-synaptic induction" (Thoenen Mueller & Axelrod 1969 Molinoff Brimijoin & Axelrod 1971)

The nicotine was given in the drinking water in this study This may be a way for chronic administration of nicotine that comes closer to that used by a smoker than injections of nicotine do even when the injections are given several times a day

Since nicotine by changing the sympathetic activity may influence blood pressure and heart rate these parameters have been followed By recording directly from implanted catheters the use of anaesthesia or handling of the animals during the registration could be avoided

METHODS

Male Sprague-Dawley rats with an initial body weight of about 200 g were used. They were kept in groups of 5 animals (at 23°C) with food and water (without or with nicotine added in different concentrations) available ad libitum. The weight of the different groups was measured twice week. Food intake and water consumption were measured at intervals.

At different time intervals during the nicotine treatment sciatic nerve crushes were performed bilaterally under ether anesthesia a.m. Lubińska (1959) 6 or 9 h later the rats were killed and the nerves dissected out and divided in 5 or 10 mm pieces proximal and distal to the crush The DBH content in the different pieces of the nerves was determined according to Nagatsu & Udenfriend (1972) as DBH activity

In some rats, ligations were also performed around the perihilar tissue of the salivary glands (gl. submaxillaris and gl. sublingualis) 6 h later the tissue proximal to the ligature was dissected out in a 4 mm long portion. This tissue, containing ligated adrenergic axons, was also assayed for its content of DBH

For recordings of arterial blood pressure and heart rate aortic catheters were implanted under pentobarbital anaesthesia as previously described (Henning 1969). Recordings were made in conscious unrestrained animals no less than 24 h after surgery Heart rate was triggered from the pressure signal The values given represent the average of two 30 min recordings in one day

RESULTS AND DISCUSSION

The effect of nicotine (added in different doses to the drinking water as nicotine base or nicotine bitartrate) on the increase in body weight was followed Nicotine treatment resulted in a dose dependent reduction of the normal weight increase (Fig 1). No difference was found in the effect between nicotine base and nicotine bitartrate when the same doses with respect to pure nicotine were used

After 2 weeks of treatment with 50 mg of nicotine per litre of the drinking water the daily consumption of water was found to be about constant around 28 ml per day This corresponds to an intake per day of about 1 4 mg of nicotine The body weight increased from about 280 g after 2 weeks of treatment to about 360 g after 6 weeks. The daily consumption of nicotine will thus be between 5 mg per kg after week 2 and 3 9 mg per kg after week 6 The one pack-a-day cigarette equivalent has been discussed to be 1 14 mg per kg and day

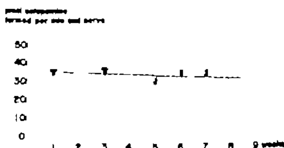


Fig 2 Dopamine-β-hydroxylase (DBH) accumulated per hour proximal to crush of the sciatic nerve of rats with or without nicotine in the drinking water DBH is expressed as DBH activity in μmol octopamine formed per minute and nerve piece. (O) indicates no nicotine in the drinking water (●) indicates 90 mg nicotine base per litre of water (□, ■ and Δ) indicate nicotine bitartrate corresponding to 25 50 and 100 mg pure nicotine per litre respectively Every point is the mean value generally of 4 to 5 estimations.

Table 1 *Mean arterial blood pressure (mmHg) and heart rate (beats/min) during two consecutive days in conscious rats with or without nicotine in the drinking water*

The values are means with S.E. and number of rats in brackets

	Heart rate		Blood pressure	
	Day 1	Day 2	Day 1	Day 2
Controls (no nicotine)	337	323	131	1.5
	8.2	16.7	4	5 7
	(11)	(5)	(11)	(5)
Nicotine (50 mg nicotine base per litre drinking water)	321	320	13	131
	7 9	10 3	3 1	6.5
	(14)	(6)	(14)	(6)

(Wenzel & Azmeh 1970) It is hard to compare man s intake of nicotine by smoking with intake of nicotine in the drinking water in these experiments but since we are dealing with different concentrations in the water from .5 mg to 100 mg per litre some of the concentrations may be relatively relevant to the nicotine consumption of a smoker

The water intake was higher in controls than during nicotine treatment After 3 weeks the consumption was constant, about 45 ml per day in controls. Based on circulatory data, blood pressure and heart rate the rats given 50 mg nicotine per litre water do not appear to have been dehydrated to any considerable extent. It is of interest to note that a marked reduction in body weight as compared to controls has been reported in rats receiving parenteral nicotine (0 4 or 0 8 mg per kg) 3 times a day (Schechter & Cook 1976) Furthermore it was observed in this study that the food intake was about the same in the two groups.

The effect of different doses of nicotine on the accumulation of DBH above a constriction of the sciatic nerve is shown in Fig. 2 As can be seen there was no clearcut effect of nicotine as compared to the controls Moreover there was no effect of nicotine on the accumulation of DBH above a constriction of the adrenergic axons to the salivary glands (data not shown)

This lack of action of nicotine on the accumulation of DBH above a constriction of adrenergic axons strongly indicates that the production of new granules was uninfluenced upon by nicotine in the doses used It appears therefore unlikely that the activity of the adrenergic neurons was changed by the nicotine Several theoretical possibilities for such a lack of effect may be discussed However it may be of more direct interest to study the effect of nicotine on the circulatory system

Table 1 shows that after 30 days of treatment with 50 mg nicotine per litre in the drinking water there was no effect of nicotine either on blood pressure or on heart rate One explanation for this lack of effect as compared to other investigators may be differences in the methods used for the registration of the circulatory parameters

When the chronic treatment with nicotine was terminated and pure water was given some of the groups showed signs of abstinence both with respect to DBH-accumulation and circulatory parameters These results will be presented elsewhere.

The results of this study show that even when nicotine is given in the drinking water in doses which retard the normal increase of body weight there were no signs of increased activity of peripheral adrenergic neurons nor were any changes in blood pressure or heart rate observed This seems to be in agreement with the clinical observations that smoking and hypertension are not imperatively correlated (for ref see Wenzel & Azmeh 1970)

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Cholinergic inhibition of sympathetic vasoconstrictor tone in the cerebrovascular bed mediated by nicotinic type receptors

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ABSTRACT The cholinergic influence on the cerebrovascular bed was studied in cats and rabbits using combination of in vitro and in vivo approaches. Isolated pial arteries were superfused with ^{3}H-norepinephrine and the efflux of tritium was studied under various conditions of transmural electrical stimulation of the perivascular sympathetic nerves. Field stimulation increased the radioactivity in the efflux in a frequency-dependent manner. The amount of tritium overflow (of which approximately 70% represents liberated radioactive norepinephrine) from the nerves during stimulation was enhanced by hexamethonium, the effect being inhibited by nicotine or acetylcholine. Thi action of nicotine and acetylcholine was counteracted by hexamethonium (but not by atropine). Carbachol infusion under in vivo conditions increased blood flow in the caudate nucleus as measured by thermoclearance. This effect was inhibited by atropine. Postganglionic stimulation of the cervical sympathetic chain above the superior cervical ganglion produced, in itself a reduced local cerebral blood flow. The response (but not the flow reduction obtained by exogenous norepinephrine) was diminished during infusion with carbachol. The inhibition amounting more than 90% of the sympathetic nerve action on CN blood flow was not affected by atropine. It is concluded that (a) there are direct cholinergic dilator mechanisms in the cerebrovascular bed mediated by muscarinic-type of cholinergic receptors in the vascular smooth musculature and (b) the perivascular adrenergic nerve terminal possess nicotinic-type of cholinergic receptors mediating an inhibition of the local norepinephrine release probably through an action by the cholinergic nerve terminals, which run parallel t and in close association with the adrenergic axons separated only by 25 nm distance

On the basis of cholinesterase histochemistry following appropriate inhibition of pseudocholinesterase it ha been shown that the pial vessels are supplied by numerous cholinergic nerves which run along with the adrenergic axons in the same strands of the perivascular autonomic plexus (Edvinsson et al 197?) Electron microscopy has demonstrated that both systems of nerve terminals form close contacts with the vascular smooth muscle cell in a manner suggesting a functioning innervation (Nielsen et al 1971) Both α-adrenergic and β-adrenergic receptors have been identified and characterized in pharmacological experiments (Edvinsson & Owman 1974) The findings that pial vessels respond to acetylcholine whose effect is blocked by atropine (Edvinsson et al 1977 Owman et al 1978) suggest the presence also of cholinergic receptors in the vascular wall The close association between apposing adrenergic and cholinergic axon terminals (Nielsen et al 1971 Edvinsson et al 1972) has indicated that the situation is complex and probably includes terminal interaction between the two nerve system (Edvinsson et al 1977) whereby liberated acetylcholine influences not only the vascular smooth musculature but also the adjacent adrenergic nerves at the sites of apposition.

METHODS

Pial artery preparations were obtained from the the brain of adult cat of either se weighing

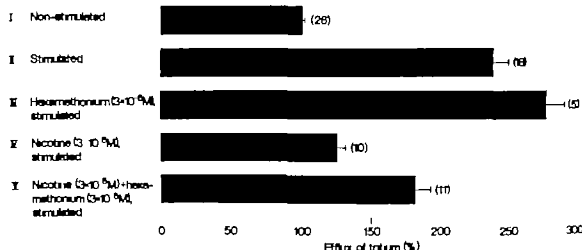


Fig 1 Measurements of tritium overflow in pial vessels. Pronounced increase during electrical field stimulation (II) in comparison with nonstimulated controls (I $p<0.001$) The increased efflux during stimulation is enhanced by hexamethonium (III $p<0.05$) and inhibited by nicotine (IV $p<0.05$ in comparison with nonstimulated controls I). The nicotine effect seen during transmural stimulation is counteracted by hexamethonium (V $P<0.01$ in comparison with IV) The mean amount of radioactivity in three 0.5-ml samples taken every minute before stimulation is set as 100%. Number of experiments within parentheses.

Sympathectomy was carried out under sodium pentobarbital (30 mg/kg, i.p) anesthesia by excision of the superior cervical ganglia at least 1 week prior to the experiments The animals were killed by bleeding under the same anesthesia, the brain was removed and the pial vessels were immediately dissected out and placed in aerated Krebs-Ringer buffer solution of the following composition (mM) NaCl 118, KCl 4.5 $MgSO_4$ 7 H_2O 1.0, KH_2PO_4 1.0 $NaHCO_3$ 25 $CaCl_2$ 2 H_2O 2.5 glucose 6.0. The tissue for studies of the norepinephrine release during mural electrical stimulation consisted of 2–3 pieces of al arteries up to 10 mm long which were pre-incubated for 20 min at 37°C in Krebs-Ringer buffer solution (aerated with 95% O_2 and 5% CO_2) containing 10^{-} M of ^{3}H-norepinephrine (labelled at the β-carbon atom 30 mCi/mg) Either intact or sympathetically denervated vessels were used. After pre-incubation they were transferred to a superfusion chamber equipped with platinum electrodes for electrical field stimulation (Edvinsson et al. 1977) according to the technique described by Farnebo (1971). The chamber was perfused with pre-warmed (37.5°C) Krebs-Ringer solution and the efflux collected (one 0.5 ml sample every minute) in liquid scintillation vials. After addition of 10 ml Instagel (Packard) counting of radioactivity was performed in Packard Tri-Carb liquid scintillator

Following incubation in the presence of H-norepinephrine, the pial vascular preparations were transferred to small chamber and superfused with buffer and the radioactivity of the overflow was measured in the efflux before and during electrical field stimulation by 12 V (measured by an oscilloscope between the electrodes in the chamber), 1 ms pulse duration and 10 Hz frequency

The basal level of tritium overflow was determined as the mean amount obtained during 3 1 min period before stimulation, and was set as 100% The ... em ... then stimulated electrically by Grass Model S44 stimulator and the efflux collected during a further period of 3×1 min for determination of a new mean level of radioactivity Three control samples were again collected without stimulation. The antagonists were added to the superfusate 15 min before (and remained present during) next stimulation period. The agonists were similarly added 5 min before. The viability of the system was checked by a final 3 min period of electrical stimulation in the absence of drugs, followed by a 3 min control period

Experiments in

Blood flow was measured in the caudate nucleus (CN) by heat clearance (Seylaz 1965 Seylaz et al. 1973 Sercombe et al 1975) The thermistor probes were implanted chronically and the animals were used 10–14 days later possible inflammation or edema had then been resorbed. PaO_2 and $PaCO_2$ were measured continuously by mass spectrometry (Seylaz et al. 1974). Arterial blood pressure (B.P) was measured in the aorta via conventional pressure transducer

Thirteen rabbits of either sex, weighing about 3 kg, were anesthetized by diazepam-pentobarbital. After tracheal cannulation careful dissection of the sympathetic trunk and superior cervical ganglion was made Pairs of stimulating electrodes were placed on the nerve trunk and the distal part of the ganglion, while a hypodermic needle, 0 3 mm d. and connected to 3 µl Hamilton syringe, was implanted int the proximal part of the ganglion. Another such needle was implanted in the common carotid artery A Teflon catheter was introduced into the aorta via the femoral artery this was used to guide into place the mass spectrometer cannula, and also to measure the blood pressure and take blood samples for analysis by polarography

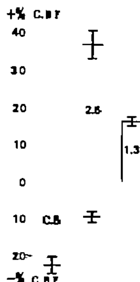


Fig. 2. Diagram summarizing cholinergic effects on local cerebral blood flow (C.B F.) measured in the CN. Open bars. Mean increase (±S E.) in CN blood flow during intracarotid infusion with 2.5 or 1.3 μg/kg/min of car bachol. Suppled bars: Mean reduction (±S.E.) in CN blood flow during postganglionic cervical sympathetic nerve stimulation (C.S), alone or during the intracarotid infusion of 2.5 μg/kg/min of carbachol

RESULTS

Experim nts in vitro

In non-sympathectomized vessels the spontaneous rate of tritium overflow during a 3-min sampling period corresponded to 2836±456 cpm (mean ± S E.) Electrical field stimulation significantly increased the radioactivity in the efflux, the effect being more pronounced with increasing frequency tested at 3 6 and 10 Hz. After cessation of stimulation the amount of radioactivity returned to a level slightly below that recorded before stimulation (cf Farnebo 1971) The increase of radioactivity in the efflux (at a st mulation frequency of 10 Hz) was abolished by the presence of 6×10^{-6} M bretylium or 10^{-6} M guanethidine in the buffer solution (Edvinsson et al. 1977)

I the presence of hexamethonium, the electrically induced o erflow of tritium was enhanced (Fig 1) The effect of electrical field stimulation was on the other hand markedly inhibited by 3×10^{-6} M acetylcholine or $3\times10^{-}$ M icotine (Fig. 1) The effect of either acetylcholine or nicotine could be counteracted by 3×10^{-6} M hexamethonium (Fig. 1) but not by atropine tested in doses up to 10^{-} M (i.e. doses which had been shown to have marked effects on the motor response of the pial vessels to acetylcholine as medicated by the muscarinic receptors Edvinsson et al 1977) The spontaneous efflux of tritium (from non-stimulated vessels) was not altered when nicotine was added to the perfusion solution in a concentration of 3×10^{-6} M

Following sympathetic denervation of the pial vessels the radioactivity in the efflux was only 292±86 cpm and it was not altered by electrical field stimulation as tested at a frequency of 10 Hz (Edvinsson et al 1977)

Exp riments in vi o

Before each test with carbachol the ganglionic nicotinic synapses were blocked by local injection of 0.5–2.0 μl of hexamethonium solution thus avoiding possible pharmacological stimulation of postganglionic neurons. Total blockade of the transmission was verified by the suppression of the effects of preganglionic stimulation on the iris and on the CN blood flow

The effect of postganglionic stimulation (after local ganglionic transmission blockade) on the CN blood flow was studied (see also Aubineau et al 1977) A large number of identical stimulations were carried out before during and after intracarotid infusion of ..5 μg/kg/min carbachol During the control stimulations a fall in flow of 22.0±2.5% (mean±S.E.) was found (Fig. 2) A few min after the beginning of the carbachol infusion the same stimulation induced a fall only 9..±1.8% (Fig.) This inhibition disappeared progressively until 5–10 min after the end of the carbachol infusion where after the effects of stimulation were as before The inhibition of sympathetic nerve action thus attained 58±20% It was not diminished by doses of atropine which almost totally inhibited the flow rise induced by carbachol The diminution of CN blood flow induced by norepinephrine injections were unchanged during carbachol perfusion and under the influence of atropine It may be noted that carbachol appeared to potentiate the rise in flow following sympathetic stimulation, whether or not at ropine was present

COMMENTS

Method logical questions regarding the validity of the superfusion technique to tudy sympathetic

nerve activity during electrical field stimulation have been discussed elsewhere (Edvinsson et al 1977)

The amount of increase in radioactivity recovered in the effluent during in vitro stimulation of the perivascular sympathetic nerves was significantly reduced by the presence of acetylcholine or nicotine the effect being counteracted by hexamethonium but not by atropine Similarly the amount of fall in CN blood flow during sympathetic nerve stimulation in vivo was reduced by infusion of carbachol an affect that was not inhibited by atropine The experiments suggest the presence of a nicotinic type of cholinergic receptors on the perivascular sympathetic nerves which was corroborated by the finding in vitro that pretreatment of the superfused brain vessels with hexamethonium enhanced the increment in tritium overflow during transmural stimulation A further indication that the site of this inhibitory action of carbachol is on the adrenergic nerve was given by the fact that the vasoconstriction obtained by systemic administration of norepinephrine was not modified during the carbachol infusion In view of the close axo-axonal relationship between the adrenergic and cholinergic nerve varicosities (Nielsen et al 1971 Edvinsson et al 1977) it is possible that the cholinergic nerve terminals can act synaptically on adjacent sympathetic nerves in the vessel wall Since the adrenergic sympathetic nerves apper to be of vasoconstrictor nature (see Owman et al 1978) it follows that the cholinergic system besides its direct (postjunctional) vasodilatory action on muscarinic receptors in the vascular smooth muscle can promote vasodilatation also indirectly through an inhibition of the norepinephrine release via the nicotinic receptors present on the perivascular sympathetic fibres

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Effects of tobacco smoking on the blood temperature during exercise

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ABSTRACT By thermometry in mixed venous blood the thermal reactions to exercise programmes of different durations were studied in healthy volunteers before and after smoking. Pulmonary oxygen uptake ($\dot{V}_{O_2}$) arterio-venous oxygen difference (AVD) and heart rate (HR) were also measured It was found that exercise-induced rise in blood temperature was considerably augmented after smoking This effect was paralleled (and probably partly caused) by an increased heat production reflected by increased $\dot{V}_{O_2}$ During short-term exercise an accelerated rise in AVD might indicate that centripetal redistribution of the total blood flow could have contributed to the thermal response by a reduced skin blood flow During long-term exercise cardiac output and heart rate increased and the stroke volume decreased in response to smoking

In studies on the blood temperature in healthy men during exercise remarkably high temperature values were sometimes noted in habitual smokers. Initiated by this observation the present study was undertaken in order to find out whether tobacco smoking per se might interfere with the thermal adaptation to dynamic exercise.

By means of a technique developed for direct thermometry in mixed venous blood (Brundin 1975 Kilbom & Brundin 1976) the thermal response to exercise was measured in healthy individuals during standardized exercise programmes of different durations. The effect of tobacco smoking was studied by repeating the exercise programme after smoking. The results were compared with those from control groups who did not smoke between the exercise periods The effects on pulmonary oxygen uptake arterio-venous oxygen difference cardiac output stroke volume and heart rate were simultaneously studied

MATERIAL AND METHODS

16 healthy volunteers, 22–40 years of age all habitual smokers, were studied They came to the laboratory in the morning after light breakfast They were not allowed to smoke for 12 h before the study All the subjects were informed of the nature purpose and possible risk involved in the study before giving their voluntary consent to participate

A teflon catheter (1.. mm outer diameter) was inserted percutaneously under local anesthesia into a brachial artery A thermistor-equipped catheter (Swan-Ganz 93-118 F Edward Laboratories) was inserted into an antecubital vein and passed to the pulmonary artery under fluoroscopic control

In 8 of the subjects, mixed venous blood temperature (PA-temp), arterio-venous oxygen difference (AVD) and heart rate (HR) were measured at rest and during two identical programmes of short-term exercise performed with 30–40 min intermission During the pause 5 subjects had to smoke cigarettes before the second exercise period was started 3 subjects served as controls and repeated the exercise programme without smoking

Exercise was performed in the supine position on a bicycle ergometer starting at a work load of 20 W The load was increased by 10 W each minute The total exercise duration was 8 min and the final work load was 90 W For none of the subjects the exercise was exhaustive or tiresome the load being far below their maximum working capacity

In 8 subjects, a steady state exercise programme was used Bicycle exercise was performed at a constant work load of 50 W for 30 min PA-temp AVD HR and pulmonary oxygen uptake ($\dot{V}_{O_2}$) were measured at rest at 17–20 min and at 27–30 min after the onset of exercise At 20–25 min 5 of the subjects had to smoke 1 cigarette without interruption of the exercise before the final measurements, 3 of the subjects served as controls by performing the exercise programme without smoking

The room temperature was kept around 23°C The collected expiration air was analysed for oxygen and carbon

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The room temperature was kept around .3°C. The collected expiration air was analysed for ygen and carbon

Table 1 *Pulmonary oxygen uptake ($\dot V_{O_2}$) arterio-venous oxygen difference (AVD) cardiac output ($\dot Q$) heart rate (HR) and stroke volume (SV) in 5 healthy volunteers at rest and during bicycle exercise at 50 W for 30 min*

Smoking of 1 cigarette at 20–25 min of exercise Values in brackets refer to control group =3 exercising without smoking Mean values ± S E

	Rest	Exercise 17–20 min		27–30 min
$\dot V_{O_2}$ ml/min	263±24 (280±15)	790±32 (828±43)	$P<0.01$	827±25 (839±39)
AVD ml O_2/l blood	35±1 (36±1)	72±3 (73±3)		69±3 (75±4)
$\dot Q$ l/min	7.53±0 63 (7 11±0.18)	11 08±0.83 (11.3 ±0 70)	$P<0.05$	1_.13±0 66 (11.26±0 61)
HR, beats/min	65±4 (70±3)	98±4 (109±4)	$P<0.005$	115±6 (112±3)
SV ml	117±8 (102±3)	113±9 (104±5)	$P<0.05$	106±7 (101±3)

dioxide content according to Scholander (1947) Blood content of oxygen and carbon monoxide was measured by means of a semi-automatic oximeter (CO oximeter model 182, Instrumentation Laboratory Inc). Cardiac output was calculated by the direct Fick method. Heart rate was followed by electrocardiographic recording. The thermistor signal from the pulmonary artery catheter was fed into an amplifier voltmeter-printer system and automatically recorded each second The thermistors were calibrated after each study with a precision thermometer (Systemteknik AB) with an accuracy of ±0 01°C (Brundin 1975)

Standard statistical method were used (Snedecor & Cochrane 1969) including the paired *t* test when applicable Data in the text, tables and figures are reported as values ± standard error of the means

RESULTS

1 *Short term exercise*

Mixed venous blood temperature (PA-temp) At rest the PA temp was within the normal range 36 8±0.2°C During exercise before smoking the blood temperature rise was similar in the control and experimental groups and similar to that previously found in other normal individuals (Brundin to be published) At 30 min after exercise the blood temperature values were not significantly different from those recorded at the initial baseline measurement. This was valid for both the experimental and the control group After smoking the total exercise-induced temperature rise was almost 50% greater than that found before smoking (Fig 1) There was no difference noted during the first minute of exercise but after that, the temperature increased considerably faster after smoking than before at all the work loads studied In the control group of 3 subjects who did not smoke between the exercise periods almost identical temperature reactions were found at the two exercise periods (Fig)

Arterio-venous oxygen difference (AVD) At rest the AVD values 39±3 ml/l were within the normal limits (cf Ekelund & Holmgren 1967) During exercise the AVD increased in the same manner as that seen in other healthy individuals (Brundin to be published) After smoking the AVD at rest fell significantly as compared with the initial baseline measurement and was now 29 ml/l±1 indicating that cardiac output was increased by smoking. No such fall in AVD was observed in the control group.

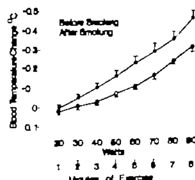


Fig 1 Mean blood temperature change during short term exercise performed by 5 healthy volunteers before and after smoking of 2 cigarettes. Bars indicat S E.

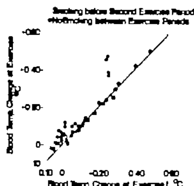


Fig 2 Comparison of individual changes in blood temperature measured during repeated short term exercise periods. 5 subjects (●) smoked 2 cigarettes between the exercise periods 3 subjects (O) did not.

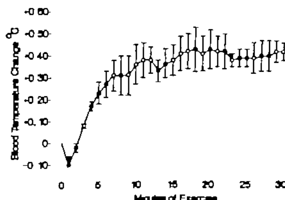


Fig 3 Mean change in blood temperature during long term exercise (50 W) performed by 3 healthy volunteers. Bars indicate S E.

During exercise after smoking, however the AVD reached values similar to those observed during the first exercise period. Thus the exercise induced increase in AVD (Δ AVD) was significantly greater after smoking. In the control group no such differ ences occurred

The carboxy-hemoglobin in the arterial blood increased by around 2% after smoking and was not significantly changed during the second exercise period.

Heart rate (HR) At rest the mean HR was 62±3 beats per min Increased to 116±10 at 90 W and was 30 min after the first exercise period 61±3 Smoking significantly increased the HR at rest to 85±6 and during the entire second exercise period the HR was significantly higher at each work load ending at 130±8 at 90 W In the control group the HR reaction was almost identical during the two exercise periods.

2. *Long-term exercise at steady state*

Pulmonary oxygen uptake ($\dot{V}_{O_2}$) At rest before exercise V_{O_2} was around 25% higher than the basal values calculated from body size During exercise at 50 W $\dot{V}_{O_2}$ was around 3 times higher than at rest. After smoking there was a further significant increase of around 5%. In the control group $\dot{V}_{O_2}$ did not increase significantly during the last 10 min of exercise (Table 1).

PA-temp rose relatively steeply during the first 5 min of exercise and continued to rise more slowly up to 12 min After that a steady state level was reached (Fig. 3) During smoking an additional temperature increase was recorded and a higher steady state level was attained at 6 min after the onset of smoking (Fig. 4) This temperature increase was about 50% of the first temperature rise. In the control group the PA-temp was not significantly higher at 30 min than at 17 min of exercise

Arterio-venous oxygen difference (AVD) showed a two-fold increase at 17 min of exercise and was not significantly changed by smoking (Table 1) In the control group the AVD change did not differ significantly from that of the experimental group.

Cardiac output calculated by the Fick equation, was significantly increased by around 10% after smoking. No similar difference was noted between 17 and 30 min of exercise in the control group (Table 1)

Heart rate (HR) reached a relative steady state level already within the first few minutes of exer

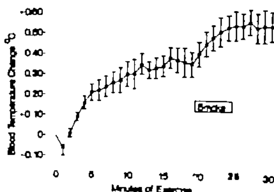


Fig 4 Effect of smoking on the mean change in blood temperature during long term exercise (50 W) performed by 5 healthy olunteers. Bars indicate S E.

cise In the control group HR continued to increase very slowly towards the end of the 30 min exercise period Smoking caused an abrupt increase in heart rate and a higher steady state value was attained during the continued exercise (Table 1)

Stroke volume (SV) was not changed by exercise per se However the markedly increased HR in response to smoking was more pronounced than the increase in cardiac output Thereby the SV was significantly reduced in response to smoking. No such effect was observed in the control group

The carboxy-hemoglobin content of the blood was significantly increased by around 1% after smoking.

DISCUSSION

The relative hyperthermia observed in the blood during exercise after tobacco smoking seems not to have been described before The effect appeared most markedly during short term exercise performed immediately after smoking. It could also be demonstrated as an increased steady state temperature level in direct response to smoking performed during a continuous period of long-term exercise

During standardized bicycle exercise the blood temperature reaction its rate of rise and its final steady state level is normally very well defined and easily reproducible (Brundin 1975 Kilbom & Brundin 1976) The results from the control groups firm that this was valid also for the actual experimental procedures. Thus the steeper blood temperature rise and increased steady state temperatures found at exercise after smoking indicate that the balance between heat generation and heat elimination was interfered with by tobacco smoking In case an increased heat generation constituted the main underlying mechanism for the relative hyperthermic reaction an increased oxygen consumption would be expected to occur in parallel The oxygen uptake could not be accurately measured during the short term exercise procedure During exercise at steady state however smoking was followed by a small but statistically significant increase of the oxygen consumption At rest nicotine has been reported to increase the metabolic rate by up to 10% (for ref see Ilebekk, Miller & Mjøs 1975) but during exercise no such effect seems to have been demonstrated earlier The increase in metabolic rate might constitute a direct metabolic response to the augmented sympatho-adrenal activity which is known to occur in response to tobacco smoking (for ref see Burn et al. 1959)

Although an increased heat generation could explain the relative hyperthermia during exercise after smoking, other mechanisms might well have contributed Thermoregulation during exercise normally depends mainly on the capacity of heat elimination from the body which especially during *short-term exercise will require an adequate increase* of skin blood perfusion. There is no reliable method available for direct measurements of the total skin blood flow However due to its low oxygen extraction from the blood skin tissue gives a very oxygen-rich venous blood the total amount of which will affect the arterio-venous oxygen difference (AVD) for the entire body The rate of rise of the AVD-curve will thus be accelerated if the skin blood flow is reduced to any considerable extent Such an acceleration was also found during short-term exercise after smoking. During steady state exercise however smoking caused no significant changes of the AVD This might suggest that smoking was able to reduce the skin blood flow during its initial adjustment to dynamic exercise but was not able to change the level it had once attained during long-term exercise

Cardiac output was found to increase significantly when smoking was performed during prolonged exercise at a steady state work load As to the regional distribution of this increased blood flow the actual study does not allow for any direct suggestions The increased adrenaline release which might follow smoking also during exercise could be expected to per se increase the cardiac output by regional flow increments in striated musculature heart splanchnic region and perhaps brain However the concomitant increase of the sympathetic nerve activity and release of noradrenaline would interfere by vasoconstrictory action and the regional net effects would not be easily predictable

The increase in heart rate observed was so marked that, in spite of the simultaneous increase in cardiac output, the stroke volume was significantly reduced when smoking was performed during the prolonged steady state exercise

It is concluded that an increased thermal response to dynamic exercise can be added to the previously known pharmacological effects of tobacco smoking. During short term exercise the

relative hyperthermia response might have been reinforced by a centripetal redistribution of the total blood flow. During long-term exercise the temperature effect was paralleled by an increased heat generation reflected by a rise in total body oxygen consumption.

This study was supported by grants from the Swedish Tobacco Company

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Effect of nicotine on the formation of prostaglandins in the rabbit kidney

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ABSTRACT The effect of nicotine on the bioformation of prostaglandins (PG) in the rabbit kidney was investigated. Rabbit kidneys were homogenized and the low-speed supernatant was incubated with ^{14}C-labelled arachidonic acid (^{14}C AA) PG precursor Nicotine was added to the incubations to produce final concentrations of $5\times10^{-}$ M to 5×10^{-4} M. Control incubations without nicotine were also performed ^{14}C-PG formed were separated and evaluated on thin layer radiochromatography I control experiments ^{14}C-labelled PGD_2, PGE_2 and PGF were formed, the relative amounts in between them being 16, 31 and 53 % respectively Nicotine dose-dependently depressed the formation of all PG formed in the kidney At $5\times10^{-}$ M the formation of ^{14}C PG was depressed to 45–80 % of control and at a concentration of 5×10^{-4} M it was depressed to 40 % or less of control It is concluded that nicotine elicits an overall depressive effect on the rabbit kidney formation of PG probably by inhibiting cyclo-oxygenation of AA.

It has been demonstrated in our laboratory that nicotine interferes with the cardiac formation of prostaglandins (PG) (Wennmalm & Justad 1975 Wennmalm 1977) In these studies nicotine facilitated the efflux of PGE from the perfused rabbit heart Later observations have indicated that nicotine selectively counteracts rabbit heart prostacyclin formation (Wennmalm 1978*a*) and in parallel increases the amount of PGE formed in this organ (Wennmalm 1978*b*). According to these ex periments nicotine would rather than accelerating cardiac PG synthesis in general induce a change in the relative amounts of the different PG formed

In the present study we have investigated the effect of nicotine in the rabbit kidney formation of PGD_2 PGE_2 and $PGF_{2\alpha}$ and report that nicotine probably acts also at another level in the bioformation of PG namely at the cyclo-oxygenation of arachidonic acid (AA)

METHODS

Rabbit of mixed sexes and strains, weighing from 0–3.5 kg, were used. They were killed by blow on the head and exsanguinated via the carotid artery The kidneys were rapidly removed, freed from fat tissue and immersed in vol of 0.1 M potassium phosphate buffer pH 7 4. They were subsequently minced and homogenised with teflon piston at 300 rpm and 5°C during 30 s. The supernatant thus obtained was used for the incubation experiments.

Supernatant corresponding to 1/7 rabbit kidney (vol 6 ml) was incubated with 1.25 µCi Na-arachidonate (prepared from ^{14}C AA New England Nuclear sp.act. 40–60 mCi/mmol) for 5 min Nicotine obtained from the chemical analytes laboratory at the Swedish Tobacco Company was dissolved in saline and kept at −18°C in the dark. Immediately before the incubation the nicotine solution was added to the incubates to produce final concentrations of $5\times10^{-}$ to $5\ 10^{-4}$ M. Control incubations, in which no nicotine was added were also performed. Incubation was interrupted by addition of 70 ml of water and adjustment of pH to 3 4

Following decontamination of the incubation the acidified incubate was extracted twice with 25 ml of ethyl acetate The organic phase was washed once with 4 ml of 0 5 M acetate buffer pH 6 5 and twice with 4 ml of water It was subsequently dried on Na_2SO After evaporation it was reconstituted in 0 ml of ethanol Radiochromatographic separation was performed using 0.25 ml DC Kieselgel Fertigplatten F 254 (Merck) in solvent ethyl acetate acetic acid 2,_ 4-trimethyl-penthane water (90 20 50 100 / water phase discarded AIX, Hamberg & Samuelsson 1966) against standards of PGD_2 PGE_2 and $PGF_{2\alpha}$ Radioscans of the chromatograms from incubations using ^{14}C AA substrate were developed with Berthold Dünnschicht Scanner

The relative amounts of the various ^{14}C-labelled prostaglandins formed were calculated from the areas under the different radiopeak in relation to the total area of the

Table 1 *Distribution of ^{14}C PG formed from ^{14}C AA in supernatant of homogenized rabbit kidney*

The value for a single PG is expressed in % of the total amount of PG formed

	PGD_2	PGE_2	$PGF_{2\alpha}$
Mean	16	31	53
S E.	2	2	10
	4	7	7

identified peaks in the chromatograms. Values in text, table and figures are presented as mean ± S E.

RESULTS

Incubation of the supernatant of the centrifuged kidney homogenate with ^{14}C Na-arachidonate resulted in formation of various labelled compounds which were readily distinguishable in the radioscans Major peaks were found in parallel to the AA, PGD_2 PGE_2 and $PGF_{2\alpha}$ standards No other radio-peaks were obtained regularly The relative quantitative distribution of the ^{14}C PG-peaks obtained in control experiments is shown in Table 1 ^{14}C $PGF_{2\alpha}$ was the major compound formed followed by ^{14}C PGE_2 and ^{14}C PGD_2.

Addition of increasing doses of nicotine to the incubates induced a progressive decrease in the amount of ^{14}C PG formed Thus the formation of ^{14}C $PGF_{2\alpha}$ which at a nicotine concentration of $5\times10^{-}$ was about 80% of control (without drug) decreased to about 40% of control at a nicotine concentration of 5×10^{-4} M The formation of ^{14}C PGE was likewise depressed from about 75% of control at $5\times10^{-}$ M to about 15% at 5×10^{-4} M The formation of ^{14}C PGD_2 was most sensitive to the inhibitory action of nicotine At 5×10^{-4} M the amount of ^{14}C PGD_2 formed was only about 45% of control and at 5×10^{-4} M no formation of ^{14}C PGD_2 was observed (Fig 1) The relative distribution of the ^{14}C PG formed varied with increasing concentrations of nicotine ^{14}C $PGF_{2\alpha}$ increased from 57% in controls to 89% at a nicotine concentration of 5×10^{-4} M and ^{14}C PGE_2 decreased from 33% in controls to 11% at 5×10^{-4} M The formation of ^{14}C PGD_2 was rapidly depressed being only 8% of the total ^{14}C PG formed at $5\times10^{-}$ M

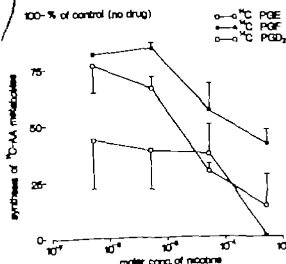


Fig 1 Supernatant of kidney homogenate incubated with ^{14}C Na-arachidonate and different concentration of nicotine. The symbols indicate mean ± S.E. of the various ^{14}C-PG in % of the amount formed in control incubations without drug.

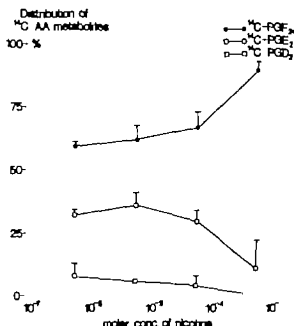


Fig 2 Distribution of the ^{14}C PG formed in supernatants of kidney homogenate incubated with ^{14}C-Na-arachidonate and various concentration of nicotine. The symbols indicate mean ± S E. of the various ^{14}C PG in % of the total amount of ^{14}C-PG formed at that concentration of nicotine

DISCUSSION

The present experiments demonstrate that increasing concentrations of nicotine elicit a dose-related inhibitory effect on the formation of ^{14}C PG formed

from ^{14}C AA in rabbit kidney homogenates. This effect of nicotine is not completely uniform. Thus the formation of PGD_2 is apparently very sensitive to the inhibitory action of nicotine the amounts of ^{14}C PGD formed being depressed by more than 50 % at a nicotine concentration of 5×10 M and completely inhibited at 5×10^{-4} M. The synthesis of ^{14}C PGE_2 is also strongly depressed by nicotine at a concentration of 5×10^{-4} M being decreased to about 15 % of control. The formation of ^{14}C $PGF_{2\alpha}$ is the most insensitive to the inhibitory action of nicotine at 5×10^{-4} M the synthesis was still about 40 % of control. These data seem to indicate a difference in sensitivity to the inhibitory effect of nicotine of the pathways from PG endoperoxides to PGD, PGE_2 and $PGF_{2\alpha}$ respectively. Such a difference appears even more evident if the distribution of ^{14}C PG formed in the presence of different concentrations of nicotine is calculated as in Fig. 2. In that diagram the relative increase in the formation of ^{14}C $PGF_{2\alpha}$ at higher concentrations of nicotine is evidently visible.

Although thus differences in sensitivity to nicotine apparently exist in the kidney formation of various PG the most significant finding of this investigation is that nicotine depresses the total amount of PG formed from AA. This strongly suggests that nicotine acts at an enzymatic level that is common to the formation of all PGs i e on the cyclo-oxygenation of AA to PG endoperoxide. The observed differences in sensitivity to nicotine of the formations of PG D E, and F probably merely reflect that the dominating metabolic pathway (leading to formation of $PGF_{2\alpha}$) on restriction of the amounts of PG endoperoxides formed retains an increasing fraction of the amount of substrate (PG endoperoxide).

In conclusion nicotine affects the formation of PG in the supernatant of rabbit kidney homogenates considerably. The various metabolic pathways for PG synthesis in this preparation display marked differences in sensitivity to the inhibitory effect of nicotine which makes the pharmacological significance of the data uncertain. In inhaling cigarette smokers the nicotine concentration in plasma has been reported to be about $5\times10^{-}$ M (Armitage et al 1974). In the current experiments that concentration produced a 50 % inhibition of the formation of PGD_2. The possibility that cigarette smoking affects PG formation in the human body therefore seems likely and deserves increased attention.

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Psychological effects of tobacco smoking

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The main purpose of the psychological studies of tobacco smoking is to elucidate the mechanisms underlying the peculiarly persistent habit of inhaling into the mouth and lungs the smoke from burning tobacco. What are the psychological och pharmacological factors interacting in the starting and maintaining of such a habit?

The complicated question of smoking motivation has been approached in many different ways. The simplest way to find out why people smoke would seem to be to ask them. However it is difficult to obtain valid information on motives by direct questions. To overcome this problem, indirect approaches have been tried. How do people rank a set of possible motives? Which of several possible changes in mood and alertness are experienced most often? In what situations or moods do people tend to smoke or feel like smoking? Another indirect approach to smoking motivation is to study the personality traits of heavy smokers. Who are the people who get addicted to smoking?

Such approaches have been used in some studies on smoking motivation performed by students in the Department of Psychology, University of Stockholm (Hultberger 1974, Waller 1975). Some of their findings will be briefly reviewed here, as a background to the main topic for the present paper—a review of some studies of objective effects of tobacco smoking on mental performance (attention or vigilance) and on psychophysiological and pain measures, interpreted in terms of arousal regulation or reward effects.

Personality traits of heavy smokers

It has been consistently found that amount of smoking tends to be associated with the personality traits of extraversion, impulsiveness and monotony avoidance (sensation-seeking), and psychopathy estimated by personality inventory scales (Schalling 1977). Impulsiveness refers to behavioural tendencies like acting on the spur of the moment, lack of anticipation or planning, and the seeking of variety and thrill. These personality traits are among the few for which biological correlates have been consistently found. Among these are indices of a low level of reticular-cortical arousal (Schalling 1978), e.g. a low frequency of spontaneous fluctuations in skin conductance and some EEG characteristics. It is possible that the smoking of heavy smokers may constitute a link in the self-regulation of arousal, one easily available method of increasing the capacity to sustain attention. This interpretation is rendered more likely by the known effects of nicotine on release of acetylcholine (Holmstedt & Lundgren 1967) which appears to be related to increased cortical arousal (Warburton 1975).

Smoking motives and subjective effects of smoking

Waller (1975) studied smoking motivation in several groups of male moderate smokers. The subjects tended to rank motives related to pleasurable and calming effects highest. The two most frequently reported effects of smoking were an increase in ability to relax and to concentrate. However, the individual variations were substantial.

The more neurotic or anxiety-prone subjects reported that they were smoking predominantly in order to achieve calmness. However, increased irritability and tension were the main *effects* reported by these subjects. These seemingly paradoxical reports may be interpreted as reflecting irrational behavior in neurotic persons. However, for persons with habitually high arousal, a further increase may lead to a supraoptimal arousal state (Eysenck 1967).

Smoking in high arousal and low arousal situations

Desire for smoking obviously varies with the situation. Applying a list of different situations, Hultberger (1974) found higher ratings for smoking desire in low arousal situations (e.g. relaxing at home) than in high arousal situations (e.g. waiting for a difficult examination) which is in line with English findings, using the same list. A classification of smokers on the basis of this list into high-

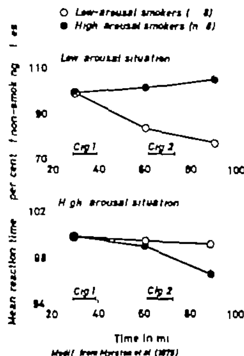


Fig 1 Mean reaction time in smoking sessions in per cent of non-smoking values for eight low-arousal smokers (open circles) and eight high-arousal smokers (filled circles) (from Myrsten et al 1975)

arousal smokers and low arousal smokers yielded interesting sex and personality differences Female subjects were predominantly high-arousal smokers male subjects low-arousal smokers The low arousal smokers had conspiciously low scores in a Psychic Anxiety scale measuring a tendency to worry and anticipate (Schalling 1977) Performance differences were reported (Fig. 1) between high-arousal and low arousal smokers selected in a similar way In line with the predictions on the basis of this classification (Myrsten Andersson Frankenhaeuser & Mårdh 1975)

However Waller (1975) using a modified situation list in which the arousal value for each item had been determined empirically by ratings found higher smoking desire in high-arousal than in low arousal situations in a group of male moderate smokers

Laboratory studies of acute effects of tobacco smoking

In a recent study from our research group the effect of tobacco smoking on *critical flicker fusion* (CFF the fusion frequency of flickering light) was measured by a new method a computerized forced-choice interactive technique (Waller & Levander 1979) This measure reflects the number of impulses that the retinal-cortical system can process in a unit of time and it is regarded as an indicator of CNS efficiency or cortical rousal Subjects were 28 male moderate smokers who participated in a Smoking (S) and a Non Smoking (NS) condition each of one hour duration. CFF was measured during 15 two min trials in each condition In the S condition the level of CFF was studied intermittently during 15 min before smoking during 15 min when CFF trials were interspersed with periods in which subjects smoked three puffs on a cigarette of their preferred brand and during 30 min after smoking.

There was a marked improvement in CFF performance with a maximum 8 min after the first puff (Fig. 2) Performance was significantly higher in the S condition compared to the NS condition from five min after the first puff to 20 min after the last puff Analysis of variance was made for CFF performance averaged over trials for a presmoking period (1) a smoking period (2) and a postsmoking period (3) There was a significant condition×period interaction ($p < 01$) In Period 1 the performance was almost identical in the S and NS condition In Period 2 performance was markedly improved in the S condition followed by a slight decline in Period 3 In the NS condition there was a slight impairment in performance over periods. Order of conditions did not affect the outcome It is of interest that the duration of the effects corresponds to the known duration of increased CNS activation after smoking as indicated by EEG The smoking induced improvement in ability to discriminate visual stimuli may be taken as an indicator of an increased level of cortical arousal

In order to explore the large individual differences in effects of smoking found in this and other studies two extreme groups were formed consisting of the subjects most and least improved in the S condition The improved group had significantly higher scores in an extraversion scale ($p < 05$)

In another laboratory study from our group *skin cond ctance* was recorded during seven successive rest periods surrounding short pain tolerance measurements. In the group of subjects who were allowed to smoke spontaneous fluctuations showed a marked increase but of very short duration (Fig 3) most pronounced during the first minute of smoking The interpretation of this preliminary finding is not clear The postganglionic mediation of skin conductance responses is cholinergic

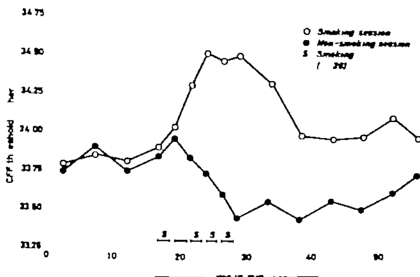
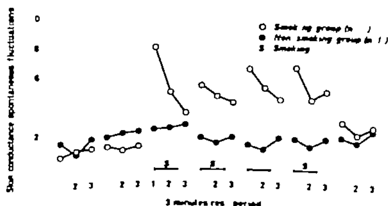


Fig 2 Mean CFF performance during 15 successiv measurements in a smoking condition and non-smoking condition ($n = 28$)

while the central and preganglionic mediation is believed to be both adrenergic and cholinergic Skin conductance spontaneous fluctuations are related to reticular stimulation in animal research (Bloch 1965) and have been associated with level of cortical arousal in human subjects (Schalling 1978). Thus the marked effects of smoking in our study may be related to an acute acetylcholine mediated phasic increase in reticular-cortical arousal

Finally some preliminary results will be given from a study of the effects of tobacco smoking on *experime tal pain response* Increased pain tolerance after smoking has been reported (Nesbitt 1973) In a recent study from our group experimental pain responses were studied in 4 subjects (8 male and 16 female) during smoking and nonsmoking conditions, using electrocutaneous stimulation (Mueser Waller Levander & Schalling 1978) Pain thresholds and tolerance levels were obtained with a method of limits (ML) Further a sensory decision analysis was made on the effects of smoking on ability to discriminate between electrical stimuli of d fferent intensity (P (A) a non-parametric meas ure corresponding to d and related to sensory



F g 1 Mean number of skin conductance spontaneous fluctuation during the first second and third minute in seven successive three minute pauses for smoking group (n 11) and non-smoking group (n 11)

components of pain) and the effects on response criteria (B a measure related to non-sensory motivational factors) There were significant sex differences in the smoking-induced change in ability to discriminate strong stimuli males being somewhat improved females impaired

A more detailed analysis was made on the 8 male subjects and an agematched group of 8 female subjects Analysis of variance on ML pain tolerance levels during the smoking and non-smoking conditions yielded a significant interaction with sex ($P<0.01$) and with scores in the Psychic Anxiety scale ($P<0.01$) Smoking increased pain tolerance levels in male subjects and independently of sex in subjects low in Psychic Anxiety

The smoking induced increase in discrimination ability in the male subjects may be related to nicotine effects on cholinergic reticular transmission systems (Warburton 1975) However the apparently paradoxical findings of an increased tolerance of pain stimulation in male subjects implies another additional mechanism of action It has been suggested that release of endorphines may be facilitated by smoking in some subjects The spectacular results of acupuncture in heavy smokers during smoking cessation therapy which have been reported also in some controlled studies (Lacroix & Besançon 1977) may imply endorphine release as one of the mechanisms behind tobacco addiction However it is obviously too early to draw conclusions and much more research is needed in these areas

Concluding comments

The mediation of the pleasure and reward-related and "analgesic" effects of smoking reported above may be pharmacologically different from the mediation of the effects on reticular-cortical arousal suggested by the CFF and skin conductance findings.

There are wide individual differences in smoking effects and an important task for the biopsychological study of smoking is to further explore these individual differences and their psychophysiological and biochemical correlates In this way it might be possible to elucidate the role of smoking in the self-regulation of arousal and mood.

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ACTA PHYSIOLOGICA SCANDINAVICA
SUPPLEMENTUM 480

Muscle fatigue in man

with special reference to lactate accumulation during short term intense exercise

By
PER TESCH

STOCKHOLM 1980

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CONTENTS

The present thesis is based on the following papers which will be referred to by their Roman numerals

I Tesch P B Sjödin and J Karlsson Relationship between lactate accumulation LDH activity LDH isozyme and fibre type distribution in human skeletal muscle Acta physiol scand 1978 103 40-46

II Tesch P B Sjödin A. Thorstensson and J Karlsson Muscle fatigue and its relation to lactate accumulation and LDH activity in man Acta physiol scand. 1978 103 413-420

III Nilsson J P Tesch and A. Thorstensson Fatigue and EMG of repeated fast voluntary contractions in man Acta physiol scand 1977 101 194-198

IV Komi P V and P Tesch. EMG frequency spectrum muscle structure and fatigue during dynamic contractions in man. Eur J Appl Physiol 1979 42 41-50

V Tesch P Fatigue pattern in subtypes of human skeletal muscle fibres Int. J Sports Med. Accepted for publication

VI Tesch P Local lactate and exhaustion. Acta physiol scand. 1978 104 373-374

VII Tesch P L. Larsson A. Eriksson and J Karlsson Muscle glycogen depletion and lactate concentration during downhill skiing Med Sci Sports 1978 10 85-90

In addition some unpublished results are included

INTRODUCTION

The feeling of discomfort and pain due to heavy muscular exercise is an experience with which most individuals are familiar Housework, occupational work leisure as well as sport activities will sometimes lead to these states which can be referred to as fatigue experience A conclusive definition of the fatigue phenomenon does not exist (cf Bartley & Chute 1947 Bartley 1965 Simonson 1971) although the question of its origin has been the subject of controversy among researchers in various scientific fields during more than half a century Mosso (1890) who constructed and used a finger ergograph was one of the first to report changes in mechanical performance during repeated muscle contractions Since then muscular fatigue as indicated by a failure to generate maximum voluntary force or a prescribed force level, has been extensively studied in order to establish its possible sites of origin (e g Lee 1906 Cobb & Forbes 1923 Reid 1928 Brown & Burns 1949 Merton 1954 Naess & Storm-Mathisen 1955 Scherrer & Bourguignon 1959 Lippold et al. 1960 Stephens & Taylor 1972 Bigland-Ritchie et al 1978)

Interest has also been focused on the behaviour of different motor unit types including their extrafusal muscle fibres in terms of fatiguability Recent review articles concerning specific characteristics of different motor units in animal (Close 1972 Burke & Edgerton 1975) as well as man (Saltin et al 1977) are available As already reviewed by Needham (1926) the classical "red" muscle possesses a greater resistance to fatigue as compared to the "white muscle. By means of mechanical definition of motor units Wachholder (1931) demonstrated that tonic muscles are more fatigue resistant as compared to phasic muscles Later this pattern has been confirmed in a number of animal studies (e g Eberstein & Sandow 1961 1963 Kugelberg & Edström 1968 Burke et al. 1971) In fact the property in this respect has been included in the fibre type nomenclature (cf Burke & Edgerton 1975) in order to distinguish between fast twitch (FT) muscle fibres with different fatiguability levels

Recently it has been possible also in man to relate sustained (Molbech & Johansen 1973 Hultén et al 1975) and repeated (Ochs et al. 1977) isometric contraction fatigue directly or indirectly to the histochemical profile of the activated muscle Using a device to study dynamic contractions (Perrine 1968) based on the force-velocity principle as introduced by Hill (1938) Thorstensson (1976) was able to demonstrate a positive relationship between the individual percentage of fast twitch (type II) muscle fibres in the vastus lateralis muscle and the decrease in force output during repeated knee extensions

Attempts have been made to explain the basic features of fatigue by relating metabolic and mechanical changes which occur during exercise (Hill & Kupalov 1930) Since the work of Fletcher & Hopkins (1907) many authors have suggested lactate accumulation as directly or indirectly causing an impaired muscular function (e g Bang 1936 Asmussen et al 1948 Karlsson 1971 Karlsson et al 1975) Some electrophysiologists who have studied changes in action potential conduction

and the frequency patterns of motor units agree on this suggestion (e g Lindström et al 1970 Mortimer et al 1970) Other experimental observations however speak in favour of a block at the neuromuscular junction (Stephens & Taylor 1972) as the site of muscle fatigue.

It is generally believed that at the onset of heavy muscular activities adenosine triphosphate (ATP) and creatine phosphate (CP) stored in the muscle exclusively will cover the energy demand Theoretically however these immediate energy sources can be depleted in man within 10 seconds (Margaria et al. 1964) Such short periods of maximal exercise concomitantly result in the formation of lactate (Pernow & Wahren 1962 Hultman et al 1967 Bergström et al 1971 Saltin et al 1971) Simultaneously signs of muscle fatigue or impairment can be recognized as indicated by a decrease in mechanical output within this time limit In order to elucidate the fatigue phenomenon, the present study was designed by combining established histochemical biochemical and electrophysiological methods when examining high intensity exercise of short duration. More specifically experiments were carried out:

a. To study physically active males with muscles differing in muscle fibre type distribution with regard to performance capacity during short term dynamic exercise of high intensity

b To analyse lactate concentration in pools of different muscle fibre types following short term intense exercise.

c To study electromyographic (EMG) patterns with emphasis on changes in frequency spectrum of motor units during exercise as described above

SUBJECTS

A total of 64 subjects was studied. Age height and weight averaged 24 (range 19-42) yrs 180 (range 170-194) cm, and 72 (range 60-89) kg They were all physically active and most were physical education students or subjects with a similar background Among these were 14 individuals participating in more regular physical training programs and competing on the national elite level in cycling long-distance running orienteering and downhill skiing In certain cases subjects were involved in more than one experiment. Major physical characteristics of the subjects are summarized in Table 1 Prior to giving their oral consent to participate in the study the subjects were informed of the purpose and the risks associated with the testing procedures The investigation was approved by the Ethical Committee at Karolinska Institutet Stockholm.

Table 1 Characteristics of subject groups Mean (±SE) values or range are given Maximal oxygen uptake was determined using exercise on a cycle ergometer

Study	n	Age yrs	Height cm	Weight kg	$\dot{V}O_2$ max ml x kg^{-1} x min^{-1}	Category
I	10	24 1	179–1	71 3±2 1	59 1±1 9	Physical education students
II	9	23–1	179–1	69 9±1 8	60 5–1 4	—
III	12	22–1	180–1	70 3±1 6	-	Physical education students Endurance trained
IV	11	25–2	179±1	72 0–1 9	59 1–1 8	Physical education students
V	23	24 1	180–1	72 2±1 7	-	Physical education students Endurance trained
VI	7	22–1	180–1	69 3–2 0	65 5±1 3	Endurance trained
VII	8	24–1	178–1	69 9±1 8	-	Physical education students
	3	20–22	174 182	72–76	-	Downhill skiers
	2	28–42	170–177	66–75	-	Ski school instructors
	8	24–1	181 1	74 2–1 5	-	Physical education students

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Muscle fatigue was expressed as the absolute as well as the relative torque decline from the initial peak torque and defined as fatigue index" A high test-retest reliability for the fatigue test has earlier been demonstrated (Thorstensson 1976) A coefficient of variation of 3 2% was obtained based on day-to-day measurements In order to evaluate whether subjects in the present study did exert MPT in the fatigue test initial peak values from a fatigue test were compared with MPT values as well as initial peak values obtained in a repeated fatigue test some days later The c v corresponded to 3 7 and 3 9% (n 22) respectively In addition 10 subjects were re-tested after 18 months During the time elapsed physical activity was maintained at an almost constant level comparable to the pre-test level. The reproducibility of the fatigue test expressed as c v was 6 9% while the corresponding value found for MPT was 5 3%

Maximal oxygen uptake (I II IV VI)

Maximal oxygen uptake was determined using leg exercise on a Monark cycle ergometer by stepwise increasing the work load according to the levelling off criterion (Åstrand & Saltin 1961) Expired air was collected in Douglas bags and the volume measured in a spirometer For O_2 and CO_2 analyses a mass spectrometer (Centronic 200 MGA, Centronic Works Croydon, England) was used. Duplicate determinations revealed a c v of 1 2% (n 20)

Cycling exercise (VI)

To make comparisons possible with previous muscle fatigue studies short term maximal cycle exercise was applied. This took place without any warm up period. An exhaustive cycling test was performed on a Monark cycle ergometer with a work load corresponding to 1?0 (range 118-121) % of $\dot{V}O_2$ max Inability to maintain a prescribed pedalling frequency (i e 70 rev x min^{-1}) was taken as criterion for exhaustion The day-to-day variation in exercise tests of this kind (n = 10) was 3 1% (c v)

Downhill skiing (VII)

To study the effect of short term heavy muscle exercise in sport events subjects performed submaximal and maximal skiing of approximately 1 min duration on a slope adjusted for competition.

The glycogen depletion pattern (Gollnick et al 1972a) was studied during two consecutive days of skiing in order to evaluate muscle fibre recruitment pattern. The exercise consisted of ski-school activity (physical-education students) and competitive slalom and giant slalom training (elite skiers) Training sessions for both groups lasted approximately 5 h per day

EMG recordings (III-IV)

To evaluate changes in electromyographic activity during the muscle fatigue experiments EMG was registered from the vastus lateralis muscle Surface electrodes (Hellige Stockholm) of 10 mm in diameter were placed over the belly of the muscle as close as possible to the site of the muscle biopsy insertion. Muscle biopsies (see later) were taken at least one week prior to EMG experiments The interelectrode distance was 10 mm in the direction of the patella-crista iliaca

Amplified EMG signals were either rectified and filtered by a single lag filter (120 ms time constant) and displayed on a UV Honeywell 2012 recorder along with the corresponding torque curve or amplified and immediately stored on magnetic tape (Philips Analog 7) with a recording speed of 380 mm x s^{-1} for further analysis Peak EMG was defined as the highest point on the rectified and filtered EMG curve while integrated EMG (IEMG) was taken as the area under the rectified and filtered EMG curve as measured by planimetry Analysis was limited to the mid-range of knee extension

Using a laboratory computer (HP 2116 C) and a data processing system (Komi & Lehtiö 1973 Viitasalo & Komi 1975) IEMG and power spectral density function (PSDF) were obtained according to Bendat & Piersol (1971) In order to demonstrate changes in PSDF mean power frequency (MPF) was computed and the relative proportions were calculated for selected bandwidths (24-48 Hz 56-88 Hz 96-128 Hz and 136-400 Hz) For further description see Viitasalo & Komi (1977)

Muscle biopsy sampling (I-VII)

Muscle tissue specimens (approximately 30-50 mg) were taken from vastus lateralis using the percutaneous needle biopsy technique (Bergström 1962) This part of the quadriceps muscle has been found to be heavily activated when performing knee extensions during isokinetic conditions (Merrifield & Dostal 1978) maximal cycling exercise (Bigland-Ritchie & Woods 1974 Henriksson & Bonde-Petersen 1974) and downhill skiing (Eriksson et al. 1976)

Biopsies for histochemistry were mounted in embedding medium (Cryoform™ Damon Needham Hts Mass) and frozen in isopentane cooled with liquid nitrogen. For biochemistry muscle biopsies were frozen directly in liquid nitrogen Samples were stored at -80°C until analysed Specimens for glycogen determinations histochemical as well as biochemical were obtained within 5 min before and after exercise Muscle biopsies for lactate assays were obtained within 3-5 s after exercise with the subjects in a sitting position while muscle biopsies after skiing were taken within 10-15 s in a supine position.

Histochemistry (I-VII)

Serial transverse sections (10 µm) were cut in a microtome at -25°C Stainings were undertaken for ATPase activity (Gomori 1941 Padykula & Herman 1955) after preincubation at pH 10 3 4 6 and 4 3 respectively (Engel 1962 Brooke & Kaiser 1970) as well as for NADH-tetrazolium reductase (Novikoff et al 1961) For classification fibres were

identified as fast twitch (FT) and slow twitch (ST) fibres (Engel 1962) and for further subgroup classification of FT fibres (Brooke & Kaiser 1970) into FTa and FTb according to the terminology proposed by Saltin et al (1977) The percentage of each fibre type was calculated from sections containing at least 200 fibres but mostly comprising 400–500 fibres Muscle fibre area was determined on NADH-tetrazolium reductase stained fibres using a cutting and weighing procedure (Thorstensson 1976) At least 10 fibres of each fibre type subjectively rated as representative for the entire transverse sections were selected for analysis The relative muscle area occupied by FT muscle fibres was calculated according to the formula: (100 x FT/ST area x % FT) x $(FT/ST \text{ area} \times \% FT + \% ST)^{-1}$ Error of methods (c v) estimated from analysis of duplicate biopsies ranged 6–11% for area and distribution variables (Table 2)

Table 2 Methodological errors (c v) of histochemical and biochemical variables Samples used for lactate assay ranged 10–35 in concentration (mmoles x kg^{-1} wet muscle) Analyses were conducted on pools of fibres ranging 10–30 µg in dry weight. Mean values were calculated from 2–3 pools of fibres and therefore each value represents a dry weight corresponding to approximately 40–80 µg

	c v %	n[2]
HISTOCHEMISTRY		
Muscle fibre type distribution[1]	6 2	28
Muscle fibre area[1]	10 5	30
Muscle fibre area ratio[1]	7 5	26
LACTATE		
ANALYSES UNDERTAKEN SIMULTANEOUSLY		
Pool to pool unidentified fibres	6 8	35
— FT fibres	8 7	35
— ST fibres	7 2	35
— divided fibres	3 6	12
Mean to mean, unidentified fibres	4 4	32
— FT fibres	4 4	12
— ST fibres	4 4	12
ANALYSES UNDERTAKEN ON TWO SUCCESSIVE DAYS		
Pool to pool divided fibres	6 7	14
Mean to mean, —	2 2	6
ANALYSES UNDERTAKEN ON DIFFERENT OCCASIONS		
Mean to mean	5 6	12

1) Duplicate biopsies

2) Duplicate determinations

To demonstrate selective glycogen depletion pattern cross-sections (16 µm) were PAS (periodic acid-Schiff) stained for glycogen (Pearse 1961) Individual fibres were subjectively rated as filled moderately emptied and exhausted using a system similar to that proposed by Gollnick et al (1972a)

Biochemistry (I II VI VII)

Metabolites were determined in mixed muscle samples as well as in separated dissected out fibres After weighing at $-25^{\circ}C$ on a Cahn electrobalance muscle biopsies (approximately 10-50 mg) were analysed for their glycogen and lactate contents by means of fluorometer technique according to Lowry & Passonneau (1972) as modified by Karlsson (1971) using a Farrand ratio fluorometer model 24 (Farrand Optical Co New York)

Analyses on separate muscle fibres were performed in the following way: after lyophilizing muscle samples (approximately 2-12 mg) were placed under a microscope and 100-200 fragments of muscle fibres were dissected out from each sample at a constant room temperature and humidity ($20^{\circ}C$ and 30% respectively) Thereafter a part of each fibre was cut off and stained for fibre type identification (Essén et al 1975a) For lactate analysis 5-13 FT and ST fibre fragments respectively were pooled and weighed on a Cahn electrobalance or Quartz fibre ultramicro balance (Rodder "E" Microtech Services Berkely) Weight of the samples corresponded to 10-30 µg Fibres were then put into test tubes before the reagent solution was added. The lactate concentration was determined according to Karlsson (1971) Each value was calculated as a mean value for the lactate concentration in 2-7 pools Comparing values obtained from conventional analysis on whole wet (Karlsson 1971) or freeze-dried (Essén 1978) samples with the present method no systematic differences were noted in terms of lactate concentration although wet sample values tended to be lower thus confirming Karlsson (1971) Other methodological errors for lactate analysis estimated from duplicate determinations are listed in Table 2 All values were converted into wet weight values assuming a water content of 77% for muscle biopsy samples (Karlsson 1971) This value for muscle biopsy water content was confirmed and no differences were obtained irrespective of whether biopsies (n 25) were taken at rest or after short term (60 s) heavy exercise However small intra- and interindividual variations in water content were present corresponding to 75-78%

In order to express lactate concentration for the entire muscle investigated the following formula was applied: Lactate concentration in FT fibres x % FT area + lactate concentration in ST fibres x % ST area Lactate dehydrogenase (LDH) activity and muscle specific LDH (M-LDH) activity for the entire muscle were calculated in a manner corresponding to lactate.

For muscle tissue enzyme studies additional dissected pools of FT and ST fibres each consisting of approximately 100 fibre fragments were homogenised in 100 µl of 0 5 M KCl Total lactate dehydrogenase (LDH) (E C. 1 1 1 27) activity in the forward reaction (pyruvate ⟶ lactate LDH_{tot}) was determined fluorometrically

(Lowry & Passonneau 1972) in the homogenate diluted 1:4 with 0 1 M Tris-HCl buffer pH 7 5 Discelectrophoresis for separating the LDH isozymes was performed according to Dietz & Lubrano (1967) on 25 µl of the homogenate The relative contribution of muscle specific LDH monomeres (% M-LDH) was determined using a densitometric scanning technique on the separated and stained LDH isozyme bands in the gels (Sjödin 1976) The activity corresponding to the muscle specific LDH (M-LDH) was then calculated (% M-LDH x LDH_{tot}) The error of method (c v) estimated from duplicate biopsies was 3 1% (n 10) (Sjödin, personal communication)

Blood analysis

Blood samples were obtained from the finger tip and analysed for lactate concentration according to the Barker and Summerson method as modified by Ström (1949)

RESULTS AND COMMENTS

Muscle fibre type distribution in vastus lateralis of men (I-VII)

Muscle fibre type distribution in the vastus lateralis muscle in 64 healthy young men averaged 49 (range 10-79) % FT fibres The relative area occupied by FT fibres averaged 52 (range 10-77) % When subjects involved in competitive physical training were excluded the values corresponded to 53 (range 29-79) % FT and 57 (range 28-77) % FT area respectively Individual FT/ST muscle fibre area averaged in the whole material 1 20 (range 0 70-1 85) and 1 24 (range 0 88-1 85) when trained subjects were excluded (Table 3)

Table 3 Mean (range) values for muscle fibre type distribution and area in the sample studied including (n = 64) and excluding 14 individuals involved in competitive physical sports

	Fibre type distribution % FT	FT fibre area 100 µm²	ST fibre area 100 µm²	FT area %	FT/ST area
n 64	49 (10-79)	64 5 (35 9-101 7)	54 4 (29 4-88 9)	52 (10-77)	1 20 (0 70-1 85)
n 50	53 (29-79)	66 8 (36 9-101 7)	55 5 (29 4-88 9)	57 (28-77)	1 24 (0 88-1 85)

Subgroup classification of FT muscle fibres was undertaken on samples from 23 subjects The mean distribution of FTa and FTb fibres was 30 (range 13–49) % and 19 (range 0–37) % respectively

Comments: The subjects were recruited among physical education students or individuals with approximately an equivalent physical activity level Fourteen athletes involved in competitive physical training were also included

The results concerning fibre type distribution and fibre area in m. vastus lateralis of man revealed a pattern in agreement with previous studies (Edström & Ekblom 1972 Gollnick et al. 1972b) Although there is a wide range in the distribution muscles of physically active though not competitive men seem to be composed of a smaller proportion of FT fibres as compared to muscles of individuals in the sedentary state as also reported by e g Gollnick et al (1972b) and Larsson (1978) Furthermore it was noted that individuals successful in endurance events displayed a more homogenous fibre type distribution characterized by a high percentage of ST fibres and a low occurrence of FTb fibres thus confirming other authors (Jansson 1975 Nygaard Jensen 1976 Jansson & Kaijser 1977 Jansson et al 1978)

Muscle strength and its relation to muscle fibre type distribution (I–V)

Peak torque attained at a relatively high speed of movement ($180^{\circ} \times s^{-1}$) either expressed per kg body weight or in absolute terms was positively correlated to the percentage of FT fibres as well as to the relative proportion of FT fibres (r 0 48–0 65 $p < 0\ 001$ n = 50) In addition peak torque was related to the absolute FT fibre area (r = 0 30–0 33 $p < 0\ 05$)

In a subgroup (n 18) peak torque at a higher angular velocity ($300^{\circ} \times s^{-1}$) was studied This speed is approximately 50% of the maximum angular velocity that could be obtained during knee extension (Thorstensson 1976) Peak torque averaged 131 Nm or 1 8 Nm x kg^{-1} b w as compared to 151 Nm or 2 1 Nm x kg^{-1} b w at $180^{\circ} \times s^{-1}$ The observed relation between the proportion of FT fibres and peak torque developed was not further strengthened by increasing angular velocity Approximately the same correlation coefficients were found when relating peak torque to percentage of FT fibres as well as to the relative area occupied by FT fibres No relationship could be demonstrated between peak torque and the relative occurrence of one or the other type of FT fibre subgroups

Comments Peak torque developed at movements of relatively high angular velocities ($180–300^{\circ} \times s^{-1}$) was found to be related to the percentage of FT fibres thus confirming Thorstensson & Karlsson (1976) Since peak torque also was related to the absolute FT fibre area but not to mean fibre area there are reasons to believe that the quality of the activated muscle i e the proportion of FT fibres is of primary importance for force development at higher angular velocities This suggestion is in accordance with recent observations by Coyle et al (1979) Consequently the quantity i e the muscle mass as such is suggested to be of secondary importance in terms of

force development at high angular velocities Even though it has been possible to correlate mean fibre area with the cross-section of the muscle by means of dator tomography (Nygaard et al 1978a Häggmark et al 1978) such an interpretation must however be taken with caution

Muscle power and fatigue and the relation to fibre type distribution (II–VI)

Decrease in peak torque was found to occur within 10 maximal muscle contractions ($p < 0\ 001$ $n = 50$) Muscle fatigue either expressed as the relative or absolute decline in peak torque after 50 contractions was positively correlated to the muscle fibre type distribution expressed as % FT ($r = 0\ 43–0\ 61$ $p < 0\ 01–0\ 001$) or % FT area (r $0\ 40–0\ 58$ $p < 0\ 01–0\ 001$) ($n = 50$) The relationship observed between fibre type distribution and relative decline in peak torque confirms the findings by Thorstensson (1976) In a subsample muscle fatigue was found to be related also to the occurrence of FTb muscle fibres (Fig 1) Approximately the same correlation coefficients were present when relating the decline in peak torque either expressing the fibre type population as above ($r = 0\ 53–0\ 57$ $p < 0\ 01$) or to the percentage of FTb fibres ($r = 0\ 55–0\ 57$ $p < 0\ 01$)

Changes in peak torque were as expected accompanied by corresponding changes in power and work. Hence decline in power and work was correlated to the percentage of FT fibres ($r = 0\ 64–0\ 75$ $p < 0\ 05–0\ 01$ $n = 12$) The amount of work produced during the first 15 contractions (20 s) was approximately 20% greater ($p < 0\ 05$) in a group of subjects (n 5) with muscles made up by a high proportion of FT fibres (mean – SE $62^{\pm}4$%) as compared to individuals (n 5) with a low proportion of FT fibres (37–3%) With additional contractions this difference became smaller and smaller After 100 contractions the cumulated amount of work was approximately equal comparing the two groups (Fig 2)

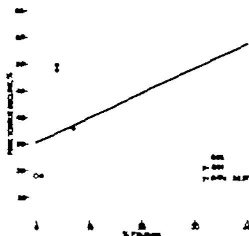


Fig 1 The 1 tionship between the percentage f FTb muscl fibres in the vastus lat ralis muscl and the decline in peak torque with 50 maximal knee extension t an angular velocity of 180° x $^{-1}$ (rad x s ^{1}) (n 23) Individual with muscl fib type distribution l s than 35% FT fibres are denot d with open (O) dots

LDH and its significance for lactate formation (I II VI unpublished results)

Correlation coefficients between LDH_{tot} as well as M-LDH activity and individual muscle fibre type distribution (% FT) were 0 96 ($p < 0 001$) and 0 98 ($p < 0 001$) respectively In addition the relative contribution of M-LDH in FT and ST fibres respectively was related to the percentage of FT fibres in the muscles investigated ($r = 0 74$ $p < 0 01$ and $r = 0 89$ $p < 0 001$) i e the proportion of FT fibres in the muscle reflected the quality of the ST fibre in respect to its LDH activity

To study further the significance of LDH enzyme activity on lactate formation capacity 10 subjects performed 25 repeated muscle contractions according to Thorstensson (1976) Mean values were 18 5 (range 3 5-31 1) mmoles x kg^{-1} wet weight (lactate) 0 90 (range 0 38-1 65) (LDH_{tot}) and 0 60 (range 0 18-1 54 (M-LDH) mmoles x kg^{-1} x min^{-1} x 10^4 wet weight The individual differences in muscle lactate accumulation after exercise were found to be related to individual variations in LDH_{tot} ($r = 0 66$ $p < 0 05$) and M-LDH ($r = 0 70$ $p < 0 05$) activity This relationship was also present in 7 endurance trained individuals At exhaustion following a short term cycling exercise the individual variation in lactate concentration (mean and range were 20 8 and 13 9-30 5 mmoles x kg^{-1} wet weight) was found to be positively correlated to the percentage M-LDH ($r = 0 73$ $p < 0 05$) (Table 4)

Comments: The data confirm earlier findings by Karlsson et al (1974) and Sjödin (1976) that muscle fibre type distribution will determine the metabolic quality of the muscle in terms of its potentials for lactate formation In addition to this an interaction appears to be present between the two muscle fibre types Thus in ST fibres of muscles rich in FT fibres the LDH activity as well as LDH isozyme pattern approached the properties of FT fibres more than in a muscle rich in ST fibres Whether or not this is an effect of adaptive mechanisms due to the environment remains speculative

Table 4 The interrelationship between some physiological histochemical biochemical and functional parameters obtained in 5 individuals performing short term high intensity exercise

Subject	$\dot{V}O_2$ max ml x kg^{-1} x min^{-1}	FT %	M-LDH %	Lactate concentration mmoles x kg^{-1} wet muscle FT	ST	whole muscle	Performance time s
I	70 0	29	39	14 4	13 6	13 8	99
II	65 9	29	45	26 2	15 5	18 8	101
III	66 6	40	39	26 7	21 4	23 8	119
IV	60 6	42	56	26 0	23 5	24 6	124
V	61 0	62	74	31 8	26 5	30 5	126

Taken together the data from the isokinetic one leg and cycling experiments seem to indicate that the activity and quality of the LDH-enzyme are critical for lactate formation and performance capacity Previously it has been demonstrated that physiological concentrations of lactate in vitro will decrease LDH activity (Karlsson et al 1974) Furthermore it has been shown that phosphofructokinase (PFK) activity considered to be the rate limiting enzyme for glycolysis is inhibited at low pH (Danforth 1965) A decrease in pH has been demonstrated concomitant to lactate formation in voluntary contracting human muscle (Sahlin 1978)

Myoelectric activity during fatiguing conditions (III IV)

Although a pronounced decrease in peak torque was demonstrated after 50 and 100 contractions corresponding to 50-75% peak EMG and IEMG decreased only slightly (n s) in the whole material studied (n = 23) In a subsample of individuals (n = 6) with muscles with a high proportion of FT fibres IEMG decreased significantly ($p < 0\ 01$) As a consequence EMG activity for the whole material either expressed as peak EMG per unit peak torque or IEMG per unit work increased progressively to level off around the 75th contraction. The change (increase) in these ratios with 50 contractions was positively correlated to individual muscle fibre type distribution expressed as per cent FT fibres ($r = 0\ 84$ $p < 0\ 001$ and $r = 0\ 78$ $p < 0\ 01$) Consequently the increase in the IEMG/torque ratio was positively related to the relative decrease in peak torque (r 0 88 $p < 0\ 001$) following 100 contractions

Different qualitative EMG changes were observed during repeated muscle contractions Thus a 25% decrease ($p < 0\ 001$) in power spectral density function (PSDF) of the EMG expressed as mean power frequency (MPF) was present in a group of individuals (n = 6) with muscles rich in FT fibres (mean - SE relative FT fibre area 67 ± 3%) In a group of subjects (n = 5) with muscles not as rich in FT fibres (43 ± 1%) a smaller nonsignificant decrease of MPF was present Intergroup deviations were noted already after 25-30 contractions ($p < 0\ 01$) When these two groups were combined, individual relative change in MPF after 25 contractions was positively related to peak torque decline (r 0 74 $p < 0\ 01$) as well as to muscle fibre type distribution expressed as per cent FT fibres (r 0 60 $p < 0\ 05$)

Comparing the frequency spectrum changes between the two groups certain differences were observed. Increase in the relative content of the lower bandwidth (24-48 Hz) as well as decrease in the higher bandwidth (136-400 Hz) were significantly greater ($p < 0\ 01$ and $p < 0\ 05$ respectively) in subjects characterized by muscles rich in FT fibres

In addition to qualitative changes in EMG activity events linked to the neuromotoric control were found to be altered with a concomitant reduction in muscle force Thus individual differences related to muscle fibre type distribution were noted when studying the time lag between the first action potential of the EMG and the corresponding torque recording at a pre-set angular velocity ($180^{o} \times s^{-1}$) (n 12) Values demonstrated initially and after 50 and 100 contractions were (mean - SE)

95±3 115±5 and 121±5 ms respectively The time lag was initally negatively related to the percentage of FT fibres (r = -0 68 p < 0 05) and the percentage time lag increase after 100 contractions was positively related to % FT (r = 0 73 p < 0 01)

Comments: The fatigue associated shift in EMG frequency spectrum towards low frequency components is in accordance with Kogi & Hakamada (1962) A significant drop in EMG activity as well as a more pronounced shift in the frequency spectrum of EMG with fatigue was noted in muscles with a high percentage of FT fibres as compared to muscles with a high percentage of ST fibres Although information regarding lactate concentration was not available from these experiments it can be assumed that after 25 contractions higher values were present in those subjects who demonstrated the most pronounced shifts in PSDF This reasoning seems to hold true since data obtained on individuals also taking part in experiments where lactate concentration was determined, revealed an inter-relationship between force decline lactate accumulation (and LDH activity) and PSDF shift (Fig 5) Whether changes in time lag between onset of EMG and torque development at a pre-set angular velocity depend on biochemical processes taking place as a result of repeated muscle contractions cannot be established here Nevertheless the present data clearly indicate that the muscle rich in FT fibres initially has the shortest time lag but suffers more during repeated contractions as compared to the muscle rich in ST fibres

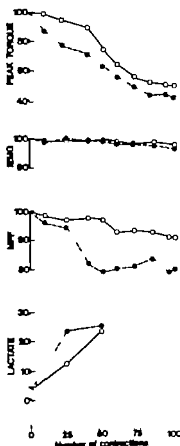


Fig 5 Cha g (expr sed X f initial v lue) f two individual in peak t rque int g ted EMG (IEMG) and mean power frequency (MPF) during repeti tion of 100 maximal kne exten ions Mus 1 biopsies f lactat oncentration (mmol s x kg^{-1} wet muscl) determinations we e obtained in separ te experiment The two subj ts diff d in respect to muscl fibre type distribution; subject GC (●): 54% FT fib s and subject GL (O): 34% FT fibre

Lactate formation during downhill skiing (VII)

In order to study the effects of acute physical activity in sports demanding great muscular power downhill skiing was used as an experimental model Low muscle glycogen values in the vastus lateralis muscle were noted after a day of skiing and in two samples studied levels below 15 mmoles glucose units x kg^{-1} wet weight were found Skilled and unskilled skiers demonstrated different selective glycogen depletion patterns In both groups a great loss of glycogen from the FT fibres was demonstrated However in the skilled skiers a pronounced depletion of ST fibres was observed as well Based on these findings it was suggested that downhill skiing to a high degree recruited the FT fibre population irrespective of training status This was supported by the fact that it was not possible to relate muscle lactate accumulation to level of proficiency However lactate concentration in the exercised muscle was positively correlated to the relative distribution of FT muscle fibres after maximal skiing (Fig 3) No such relationship was demonstrated following submaximal skiing conditions resulting in significantly lower lactate values Furthermore the FT/ST lactate ratio reached higher values during maximal skiing in individuals rich in FT fibres as compared to their opposites (r 0 84 $p < 0\ 05$)

Comments: Low muscle glycogen levels were demonstrated after skiing and such values are only reported after prolonged intense running cycling or cross-country skiing Thus it can be concluded that downhill skiing competitive as well as recreational is performed with an enhanced utilization of glycogen especially in FT fibres and that the site of the quadriceps muscle chosen is representative to study muscle metabolism during downhill skiing

Downhill skiing is evidently a physical activity which is dependent on recruitment of FT fibres irrespective of training status and is therefore in some respects comparable to the different laboratory tests applied in the present study Accordingly it suggests that the FT fibres were more active in formation of lactate It can be suggested that downhill skiing especially in less skilled skiers will be characterized by a high degree of isometric muscle exercise This assumption is supported by the fact that a high FT/ST lactate ratio was observed in subjects rich in FT fibres which is in accordance with findings obtained during standardized isometric exercise at different tension levels (Tesch & Karlsson 1977)

The present data indicated that skilled skiers recruited relatively more of their ST fibre population than unskilled. Nygaard et al. (1978b) observed pronounced glycogen depletion of FTb fibres in one inexperienced skier while none or minor loss of glycogen from these types of fibres was observed in more advanced skiers This provides further evidence of a contrasting recruitment pattern in skilled and unskilled individuals

GENERAL DISCUSSION

Metabolic profiles of muscle fibre types in man

Studies on separated human skeletal muscle fibre types disclose a consistent pattern regarding their metabolic profiles Thus in terms of metabolic potentials the ST fibre is suited for use in prolonged activities with reliance upon oxidative metabolism, indicated by higher activity of enzymes involved in the citric acid cycle e g succinate dehydrogenase (Essén et al 1975a Henriksson & Reitman 1976) as well as a LDH isozyme pattern favouring lactate oxidation (Sjödin 1976) Furthermore observations on habitually active men have indicated a greater blood flow in muscles rich in ST fibres as compared to muscles rich in FT fibres (Juhlin-Dannfelt et al 1979) Thus a relationship between leg vascular resistance and muscle fibre type distribution was demonstrated which fits well with the greater capillary density of muscles rich in ST fibres (Andersen 1975 Andersen & Henriksson 1977) Moreover a relationship between the mitochondrial content and number of capillaries surrounding each fibre has been demonstrated (Brodahl et al 1977) The FT fibre on the other hand in addition to superiority in terms of contractile characteristics (Thorstensson et al 1977) display higher activity of enzymes thought to be rate limiting for glycolysis i.e phosphorylase (Piehl & Karlsson 1977) phosphofructokinase (Essén et al 1975a Henriksson & Reitman 1976) and lactate dehydrogenase (Sjödin 1976 Thorstensson et al. 1977) Even in terms of stored metabolites differences are present between the two main fibre types Slightly higher CP concentrations were noted in FT fibres while no differences in ATP concentration of fibre types were noted in the resting muscle (Essén 1978) Triglyceride content is significantly higher in ST fibres (Essén et al 1975a) whereas glycogen content seem to be approximately equal in different fibre types (Essén et al 1975a Piehl & Karlsson 1977)

In addition the subgroups of FT fibres are known to differ in terms of enzymatic and vascular profile In most respects the FTa fibre seems to represent an intermediate type in comparison to the ST and FTb fibre (cf Saltin et al 1977)

Muscle fibre type distribution as determined by staining for myofibrillar ATPase in the muscle reflects the acto-myosin ATPase activity which in turn is assumed to be correlated with intrinsic speed of muscle shortening (Bárány 1967 Bárány & Close 1971) in the investigated muscle Considering this the present results clearly demonstrate that individual fibre type distribution to a certain extent will be important for anaerobic performance capacity as indicated by repeated muscle contractions of high intensity

Muscle fibre recruitment

Established methods are available for evaluation of the muscle fibre recruitment pattern in vivo The degree of glycogen depletion in different fibre types by histochemical means is one method to evaluate whether a certain type of fibre has been metabolically active during exercise (Gollnick, Piehl ... see scan)

Gollnick and co-workers (Gollnick et al 1973a b 1974a b) found with this approach that ST fibres seem to be recruited at muscle contractions calling for less than 20% of maximal voluntary isometric contractions At tensions exceeding 20% FT muscle fibres are recruited in addition. In dynamic (cycling) exercise with an energy need below maximal oxygen uptake ($\dot{V}O_2$ max) ST muscle fibres are the first to loose glycogen. At supramaximal load corresponding to 120% of $\dot{V}O_2$ max muscle glycogen depletion pattern indicates an initial loss from FT fibres although there are reasons to believe that a major portion of the ST fibre population is recruited simultaneously The rate of pedalling (30-120 rev x min^{-1}) does not influence the glycogen depletion pattern. Moreover at intensities close to or above $\dot{V}O_2$ max both FT fibre types are recruited (Andersen & Sjögaard 1976) However it is not possible to establish with this approach whether certain muscle fibre types are continuously involved during an entire pedal thrust or engaged only in short bursts Using the same experimental isokinetic exercise protocol as in the present study contraction velocity was found to influence muscle fibre recruitment pattern suggesting a greater involvement of FT fibres at faster velocities (Lesmes et al 1979)

Intramuscular substrates other than glycogen may have been utilized as indicated by Essén (1978) analysing single muscle fibres for their metabolic contents Furthermore a higher energy consumption per unit isometric tension developed has been demonstrated for FT as compared to ST fibres of hamster (Goldspink et al 1970) which may obscure the recruitment pattern as indicated by glycogen staining intensities In line with this is the finding that the rate of heat production in human skeletal muscles is 6 times higher in FT as compared to ST fibres (Bolstad & Ersland 1978) The rate of glycogen breakdown is also found to partly be dependent on individual muscle fibre type distribution (Juhlin-Dannfelt et al. 1977) and consequently care must be taken when evaluating for fibre recruitment pattern using the glycogen depletion method

In order to evaluate the orderly recruitment pattern of single motor units electromyography using fine needle electrodes (Hannerz 1974) and bipolar surface electrodes (Gydikov & Kosarov 1974) has been studied. Information on muscle fibre recruitment pattern in man has been obtained in isometric (cf Gydikov & Kosarov 1974 Grimby & Hannerz 1977) but also in isotonic (Grimby & Hannerz 1977) leg exercise The results confirm the size principle theory based on experiments on decerebrated cat experiments (Henneman et al 1965) In addition, an initial selective recruitment of FT motor units was demonstrated during ballistic movements irrespective of muscle tension level (cf Grimby & Hannerz 1977) These results indicate that an exclusive pool of FT motor units always will fire at onset of a muscle contraction when muscle tension is rapidly increased and/or forceful. FT fibres were also observed to be activated upon sudden and vigorous contractions in partially denervated human muscles (Warmolts & Engel 1972)

Muscle exercise and metabolism

The present findings indicate that it is possible to objectively detect a decrease in the individual s ability to produce maximum muscle force within 10 muscle contractions For such a short period of intense exercise no experimental data are available showing complete depletion of the immediate energy stores A certain reduction of muscle ATP and CP depots are however present after 10-15 s of heavy exercise (Hultman et al. 1967 Bergström et al 1971 Saltin et al 1971)

The question may be raised whether or not a complete lack of ATP in the myofibrillar regions is possible Based on frog and rat experiments impairment of contractile function due to inadequate availability of myofibrillar ATP has been suggested by e g Fitts & Holloszy (1976 1977) Murphy (1966) stimulating frog sartorius muscles demonstrated a close relationship between tension output and the ATP content of a metabolically inhibited muscle However he concluded that it is unlikely that any ATP dependent limitations will be present in the mechanical response of muscles with intact ATP restitution mechanisms

Spande & Schottelius (1970) stimulated isolated mouse muscle to fatigue and observed a linear relationship between CP concentration in the muscle and tension developed in an isometric tetanus They proposed that CP depletion can be the underlying cause of fatigue Whether the contractile machinery was directly affected or not could not be established from their results

Information concerning the glycogen depletion pattern during brief exercise in the order of 10-25 contractions are not available but most probably muscle contractions irrespective of contraction velocity and tension output must be repeated for longer periods of time in order to exhaust certain fibres (Andersen & Sjøgaard 1976 Secher & Nygaard Jensen 1976 Essén 1978)

It is open to speculation whether exhaustion following supramaximal cycling exercise is partly attributed to substrate depletion of highly recruited FT fibres By repeating exhaustive supramaximal one minute bouts approximately 50% of the FTb fibre population was found to be exhausted of their glycogen content already after 5 bouts (Thomson et al 1979) Since this fibre type to a high extent seems to contribute to the force applied at each pedal thrust (see above) selective substrate depletion cannot be excluded as being a limiting factor in intense cycle exercises in the order of 1-2 min duration.

Force generation and motor unit activity

In order to study the origin of fatigue changes in EMG activity and muscle force have been followed during various type of activity resulting in impairment of muscle function. During sustained constant submaximal isometric contraction increased EMG activity occurs (Merton 1954 Edwards & Lippold 1956 Lippold et al 1960 Kuroda et al 1970 Viitasalo & Komi 1977) This has been assumed to be due to recruitment of additional motor units and/or increased firing frequency to compensate for failure in contractility of already recruited fibres (Lippold et al 1960) In experiments

where the fatigue mechanism was studied in sustained maximal isometric contraction in man a fall in EMG activity concomitant to a loss in muscle force was observed (Stephens & Taylor 1972) The unchanged electrical activity to tension (E/T) ratio was taken as indicative of fatigue at the neuromuscular junction Ochs et al (1977) demonstrated also a drop in electrical activity when studying EMG and force relationship during rapid repetitive maximal isometric plantar flexion In comparison with Stephens & Taylor (1972) muscle tension was however found to decrease at a higher rate as compared to the EMG activity which implies an increased E/T ratio Hence muscle fatigue was indicated to be located peripheral to the neuromuscular junction.

Dissimilarities regarding changes in E/T ratio during development of muscle fatigue may be influenced by factors such as type of contractions investigated (e g submaximal vs maximal isometric contraction isometric vs dynamic contraction slow vs fast dynamic contraction) It has also been suggested that migration of activity may occur within a muscle as well as from one muscle to another (Lippold 1955 Komi & Viitasalo 1976)

Studying human single motor units a decrease in firing frequency (Marsden et al. 1971) has also been noted concomitantly with a fall in muscle tension (Gydikov & Kosarov 1974) indicating that EMG might fall without any neuromuscular block. Contradicting findings regarding E/T ratio and conclusions in the literature might be due to differences in type of motor units dominating in the investigated muscle By comparing the EMG activity of the human gastrocnemii and soleus known to differ in terms of histochemical properties (Gollnick et al 1974c Edgerton et al 1975) Ochs et al. (1977) observed a more pronounced increase in E/T ratio of the gastrocnemii muscle during repeated plantar flexions EMG studies of human dorsal interosseous muscles have shown that FT motor units fatigue more easily than ST units In addition a broader range in fatiguability was observed for FT motor units as compared to ST motor units (Stephens & Usherwood 1975) Decrease in the EMG activity during maximal effort is suggested to be due to inhibition of some motor units and/or changes in the form of motor unit potential e g amplitude duration frequency spectrum. Support for the latter possibility was demonstrated in experiments leading to conditions referred to as ischaemic (Mortimer et al 1970) In agreement with human experiments (Stålberg 1966) a decrease in the conduction velocity of stimulated cat muscle fibres was observed, which was suggested to account for observed changes in the EMG frequency spectrum. The decrease in conduction velocity was more pronounced in the gastrocnemii as compared to the soleus muscle The authors suggested lactate as responsible for these changes although no data were obtained on lactate concentration

Lactate accumulation and muscle fatigue

One of the basic findings in the present study was the higher rate of lactate formation observed in FT as compared to ST fibres The results agree with the data obtained in animal experiments (Ogata 1960 Beatty et al 1963) and previously suggested by Fletcher (1914) A lactate "flow" from FT to ST fibres may have occurred as discussed by Essén et al (1975b) mediated by membrane bound M-LDH as suggested by Sjödin (1976) and thus partly explaining the lactate content in ST fibres

Concomitant with a reduction in ATP and CP stores there is an increased lactate formation (Hultman et al 1967 Karlsson 1971) Lactate accumulation depends on lactate production and release As demonstrated lactate release in man is found to be maximal already at muscle lactate concentrations corresponding to approximately 5 mmoles x kg^{-1} wet muscle (Jorfeldt et al 1978) The authors did not indicate whether the assumed translocation hindrances for lactate were related to individual muscle fibre type distribution. Following submaximal cycling exercise greater muscle/blood lactate gradients were observed in individuals with muscles rich in FT fibres as compared to what was found in individuals with muscles rich in ST fibres (Graham et al 1978) Accordingly the present results suggest that individuals with muscles rich in high oxidative (ST and/or FTa) fibres have a greater release most probably due to a more developed capillary network (Andersen 1975 Andersen & Henriksson 1977) This suggestion is in agreement with the findings by Folkow & Halicka (1968) comparing "red and "white muscles of cat with respect to blood supply capillary surface area and oxygen uptake at rest and during exercise

A causal relationship between muscle fatigue and lactate accumulation has been suggested (Asmussen et al 1948 Karlsson 1971 Klausen et al 1972) Support for this view has been found in human experiments where performance capacity of longer duration (3–5 min) was found to be negatively affected by elevated blood and muscle lactate levels induced by previous exercise of other muscles (Karlsson et al 1975) Accordingly the same lactate concentrations were demonstrated during maximal exercise at various ambient pressures concomitant to increased performance time with increased ambient pressure (Linnarsson et al 1974)

In muscle biopsy specimens a close relationship between pH and lactate concentration immediately at termination of exercise has been demonstrated (Sahlin 1978) and the same muscle vein pH was demonstrated on exhaustion during maximal exercise at varying ambient pressures (Kaijser 1970) indicating that increased acidity in the muscle may determine the point of exhaustion The mechanism for lactate fatigue is however not clear A mechanism for high H^+ concentrations to cause impaired muscle function has been demonstrated by Fuchs et al (1970) and Nakamura & Schwarz (1972) They showed that an increase in the H concentration decreases the number of calcium ions bound to troponin during the excitation-contraction coupling This would reduce the number of active actin-myosin interactions thus decreasing contractile force Experiments on skinned frog muscle fibres have supported this hypothesis (Robertson & Kerrick 1979) Thus intracellular acidosis depressed mechanical function at the

actomyosin interaction level In addition a lowered pH was observed to require a higher Ca^2 concentration to result in a given level of activation. Eberstein & Sandow (1963) have published evidence indicating that the functional site of fatigue is in the link that couples excitation to contraction either reducing the intensity of the excitation-contraction link or decreasing the sensitivity to activation Changes in flux of calcium were suggested to alter the operation of excitation-contraction link

Recently Fitts & Holloszy (1976 1978) were able to demonstrate a linear relationship between twitch and tetanic tension and lactate concentration of stimulated frog sartorius muscle During 30 s of recovery however contractile twitch tension and ATP concentration increased rapidly while no significant reduction in lactate concentration occurred during this period In experiments where cat gastrocnemii and soleus muscles were stimulated to contract a discrepancy in fatigue pattern was demonstrated between muscles in accordance with Kugelberg & Edström (1968) Hammarberg & Kellerth (1975) A 10 s pause in stimulation was found to result in a complete recovery in contractile strength of the gastrocnemii muscle The soleus muscle on the other hand displayed a less pronounced recovery from fatigue A more rapid restitution of ATP due to higher activity of creatine phosphokinase (Burke & Edgerton 1975) in the fast contracting gastrocnemii muscle was suggested to explain in part the discrepancy in recovery pattern. In preliminary experiments using the fatigue test described here a rapid restitution of contractile tension was observed during the first 15 s of recovery from exercise in spite of unchanged or slightly decreased lactate levels Due to limited number of subjects studied the influence of fibre type distribution could not be elucidated.

Central versus peripheral fatigue

"Muscle fatigue is a complex phenomenon with numerous possible sites of origin. "Central fatigue in contrast to "peripheral fatigue was suggested already in 1903 by Setchenov and even though Bigland-Ritchie et al (1978) calculated that "central fatigue may account for 30% of total force loss in sustained isometric contractions of the human quadriceps muscle the phenomenon was not observed during the initial phase of contraction. Hence force produced through voluntary contractions and contractions stimulated via the femoral nerve decreased to a similar extent during the first 30 s of contraction. One cannot preclude that changes in the "milieu interne of the activated muscle via sensory receptors and their afferents will result in a central inhibition as has been suggested (e g Reid 1928 Lippold et al 1960 Asmussen & Mazin 1978a) When again considering experiments originally described by Setchenov (1903) Asmussen & Mazin (1978a b) it was demonstrated that exhausted individuals in a second bout of exercise were able to perform more work if diverting mental or physical activity were introduced in the pause instead of only rest. The observation was considered to be due to a change in the balance between the outflow

of inhibitory and facilatory impulses to the central nervous system implicating that central inhibition might influence mechanical output during voluntary contraction

Whether or not central fatigue occurs failure at or distal to the neuromuscular junction must be considered mainly to limit the extent of voluntary contraction. Thus muscle contraction impairment may arise from failure of transmission at the neuromuscular junction action potentials which fail to propagate or from disturbances in calcium uptake release or binding capacity due to internal metabolic changes concomitantly affecting the coupling processes

SUMMARY

1 Physically active young men were studied in terms of muscle fibre type distribution in the vastus lateralis muscle and performance capacity during various types of short term exercise The percentage of fast twitch (FT) muscle fibres averaged 49 (range 10-79) % in the studied muscle

2 Muscle force (torque) was recorded during repeated isokinetic knee extensions The absolute as well as relative torque decline was taken as criterion for muscle fatigue and was found to be more pronounced in individuals with muscles rich in FT and/or FTb muscle fibres

3 Electromyography (EMG) was recorded from the vastus lateralis using surface electrodes It was analysed for integrated EMG (IEMG) and power spectral density function (PSDF) In contrast to IEMG a general decrease in mean power frequency (MPF) was observed during repeated muscle contractions This shift was due to a decrease in the high frequency bandwidth (associated with FT motor units) of EMG with a simultaneous increase in the bandwidth of low frequency components In individuals characterized by a high percentage of FT fibres MPF decreased to a higher extent as compared to individuals with a high percentage of ST fibres

4 Lactate concentration and the activity of LDH_{tot} and M-LDH were analysed in pools of different muscle fibre types After various types of exercise (25 isokinetic contractions supramaximal cycling exercise downhill skiing) lactate concentration was related to % FT fibres in the activated muscle Lactate concentration was also related to LDH_{tot} and M-LDH activity This seems to indicate a higher rate of lactate production in FT fibres as compared to ST fibres

5 After 25 maximal repeated contractions a relationship between force decline and FT/ST lactate ratio was demonstrated A similar relationship was also demonstrated for LDH_{tot} and M-LDH ratios indicating a close relationship between the metabolic profile of the different fibre types muscle metabolism and muscle function

6 Lactate accumulation and associated metabolic changes interfering with the contractile mechanism is suggested to be responsible for muscle fatigue in the situations described here

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SUPPLEMENTUM 480

Muscle fatigue in man
with special reference to lactate accumulation during short term intense exercise

By
PER TESCH

STOCKHOLM 1980

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The present thesis is based on the following papers which will be referred to by their Roman numerals

I Tesch, P B Sjödin and J Karlsson Relationship between lactate accumulation LDH activity LDH isozyme and fibre type distribution in human skeletal muscle Acta physiol scand 1978 103 40-46

II Tesch P B Sjödin A. Thorstensson and J Karlsson Muscle fatigue and its relation to lactate accumulation and LDH activity in man Acta physiol scand. 1978 103 413-420

III Nilsson, J P Tesch and A. Thorstensson Fatigue and EMG of repeated fast voluntary contractions in man Acta physiol scand 1977 101 194-198

IV Komi, P V and P Tesch. EMG frequency spectrum muscle structure and fatigue during dynamic contractions in man. Eur J Appl Physiol 1979 42 41-50

V Tesch P Fatigue pattern in subtypes of human skeletal muscle fibres Int J Sports Med Accepted for publication

VI Tesch P Local lactate and exhaustion Acta physiol scand 1978 104 373-374

VII Tesch P L. Larsson A. Eriksson and J Karlsson Muscle glycogen depletion and lactate concentration during downhill skiing Med Sci Sports 1978 10 85-90

In addition some unpublished results are included.

INTRODUCTION

The feeling of discomfort and pain due to heavy muscular exercise is an experience with which most individuals are familiar. Housework, occupational work, leisure as well as sport activities will sometimes lead to these states, which can be referred to as fatigue experience. A conclusive definition of the fatigue phenomenon does not exist (cf. Bartley & Chute 1947, Bartley 1965, Simonson 1971) although the question of its origin has been the subject of controversy among researchers in various scientific fields during more than half a century. Mosso (1890) who constructed and used a finger ergograph was one of the first to report changes in mechanical performance during repeated muscle contractions. Since then, muscular fatigue, as indicated by a failure to generate maximum voluntary force or a prescribed force level has been extensively studied in order to establish its possible sites of origin (e.g. Lee 1906, Cobb & Forbes 1923, Reid 1928, Brown & Burns 1949, Merton 1954, Naess & Storm-Mathisen 1955, Scherrer & Bourguignon 1959, Lippold et al. 1960, Stephens & Taylor 1972, Bigland-Ritchie et al. 1978).

Interest has also been focused on the behaviour of different motor unit types including their extrafusal muscle fibres, in terms of fatiguability. Recent review articles concerning specific characteristics of different motor units in animal (Close 1972, Burke & Edgerton 1975) as well as man (Saltin et al. 1977) are available. As already reviewed by Needham (1926) the classical "red" muscle possesses a greater resistance to fatigue as compared to the "white" muscle. By means of mechanical definition of motor units, Wachholder (1931) demonstrated that tonic muscles are more fatigue resistant as compared to phasic muscles. Later this pattern has been confirmed in a number of animal studies (e.g. Eberstein & Sandow 1961, 1963, Kugelberg & Edström 1968, Burke et al. 1971). In fact the property in this respect has been included in the fibre type nomenclature (cf. Burke & Edgerton 1975) in order to distinguish between fast twitch (FT) muscle fibres with different fatiguability levels.

Recently, it has been possible also in man to relate sustained (Molbech & Johansen 1973, Hultén et al. 1975) and repeated (Ochs et al. 1977) isometric contraction fatigue directly or indirectly to the histochemical profile of the activated muscle. Using a device to study dynamic contractions (Perrine 1968), based on the force-velocity principle as introduced by Hill (1938), Thorstensson (1976) was able to demonstrate a positive relationship between the individual percentage of fast twitch (type II) muscle fibres in the vastus lateralis muscle and the decrease in force output during repeated knee extensions.

Attempts have been made to explain the basic features of fatigue by relating metabolic and mechanical changes which occur during exercise (Hill & Kupalov 1930). Since the work of Fletcher & Hopkins (1907) many authors have suggested lactate accumulation as directly or indirectly causing an impaired muscular function (e.g. Bang 1936, Asmussen et al. 1948, Karlsson 1971, Karlsson et al. 1975). Some electrophysiologists who have studied changes in action potential conduction velocity

and the frequency patterns of motor units agree on this suggestion (e g Lindström et al 1970 Mortimer et al 1970) Other experimental observations however speak in favour of a block at the neuromuscular junction (Stephens & Taylor 1972) as the site of muscle fatigue

It is generally believed that at the onset of heavy muscular activities adenosine triphosphate (ATP) and creatine phosphate (CP) stored in the muscle exclusively will cover the energy demand Theoretically however these immediate energy sources can be depleted in man within 10 seconds (Margaria et al 1964) Such short periods of maximal exercise concomitantly result in the formation of lactate (Pernow & Wahren 1962 Hultman et al 1967 Bergström et al 1971 Saltin et al 1971) Simultaneously signs of muscle fatigue or impairment can be recognized as indicated by a decrease in mechanical output within this time limit In order to elucidate the fatigue phenomenon, the present study was designed by combining established histochemical biochemical and electrophysiological methods when examining high intensity exercise of short duration More specifically experiments were carried out:

a. To study physically active males with muscles differing in muscle fibre type distribution with regard to performance capacity during short term dynamic exercise of high intensity

b To analyse lactate concentration in pools of different muscle fibre types following short term intense exercise

c. To study electromyographic (EMG) patterns with emphasis on changes in frequency spectrum of motor units during exercise as described above

SUBJECTS

A total of 64 subjects was studied Age height and weight averaged 24 (range 19–42) yrs 180 (range 170–194) cm, and 72 (range 60–89) kg They were all physically active and most were physical education students or subjects with a similar background. Among these were 14 individuals participating in more regular physical training programs and competing on the national elite level in cycling long-distance running orienteering and downhill skiing In certain cases subjects were involved in more than one experiment Major physical characteristics of the subjects are summarized in Table 1 Prior to giving their oral consent to participate in the study the subjects were informed of the purpose and the risks associated with the testing procedures The investigation was approved by the Ethical Committee at Karolinska institutet Stockholm.

Table 1 Characteristics of subject groups Mean (SE) values or range are given Maximal oxygen uptake was determined using exercise on a cycle ergometer

Study	n	Age yrs	Height cm	Weight kg	$\dot{V}O_2$ max ml x kg^{-1} x min^{-1}	Category
I	10	24–1	179–1	71 3±2 1	59 1–1 9	Physical education students
II	9	23–1	179–1	69 9±1 8	60 5–1 4	—
III	12	22±1	180±1	70 3–1 6	-	Physical education students Endurance trained
IV	11	25–2	179–1	72 0±1 9	59 1±1 8	Physical education students
V	23	24±1	180±1	72 2±1 7	-	Physical education students Endurance trained
VI	7	22–1	180±1	69 3–2 0	65 5–1 3	Endurance trained
VII	8	24±1	178–1	69 9±1 8	-	Physical education students
	3	20–22	174 182	72–76	-	Downhill skiers
	2	28–42	170–177	66–75		Ski school instructors
	8	24–1	181±1	74 2–1 5	-	Physical education students

METHODS

Statistics

Ordinary statistical methods were used to calculate mean SE and linear correlation coefficient (r) When useful intra- and interindividual differences and differences between means were tested for significance using student s "t test Calculation of error of methods was conducted on duplicate analyses and expressed as the coefficient of variation (C.V = SD of the difference between double values $x \frac{1}{\sqrt{2}} x \frac{100}{\bar{x}}$)

Force measurement (I-V)

In order to examine the early onset of muscle fatigue force (torque) development during repeated knee extensions was recorded using isokinetic equipment (Cybex II Lumex Inc New York) The principles of the apparatus have been described in detail elsewhere (e.g Hislop & Perrine 1967 Thistle et al. 1967) The reliability and validity of the apparatus are high as reported by Moffroid et al (1969) and Thorstensson (1976) The subjects were firmly fixed in a sitting position in a specifically constructed chair The lever arm of the dynamometer length held constant was attached to the lower left leg of the subjects (For further information concerning experimental set-up see Thorstensson 1976) Torque was measured at pre-set constant angular velocities corresponding to 180 and $300^{\circ} \times s^{-1}$ (π and 5 π/3 rad x s^{-1}) respectively

Peak torque of the total torque curve reproduced by a UV-recorder was measured The curve represented a motion range from 100° (0 55 π) to fully extended knee joint (0°) Work was determined as the area under the torque curve and measured with a planimetrical technique from the recording paper Power was calculated as work divided by the contraction time

The individual maximal peak torque (MPT) was defined as the highest value attained from two single attempts with 30-40 s of recovery in between Coefficient of variation (c v) when comparing torque developed in two consecutive attempts was 2.1% at $180^{\circ} \times s^{-1}$ as compared to 6 0% at $300^{\circ} \times s^{-1}$ (n 18)

When studying muscle fatigue maximal knee extensions at $180^{\circ} \times s^{-1}$ were repeated 25 50 and 100 times respectively Each contraction phase lasted 0 5 s and the following recovery period approximately 0 7 s Thus a complete contraction cycle corresponded to 1 2 s No systematic variations were observed in the time variables during one single experiment or between experiments Performance times for the fatigue experiments were then 30 60 and 120 s Experiments comprising 100 contractions at $300^{\circ} \times s^{-1}$ lasted approximately 80 s

With the exception of study III the highest value obtained during the initial five contractions was compared with the mean value of any three consecutive contractions during the entire test (i e 23-25 48-50 etc) In study III the peak torque of one contraction was substituted by the mean of the three initial contractions as criterion for the peak torque of the experiments

Muscle fatigue was expressed as the absolute as well as the relative torque decline from the initial peak torque and defined as fatigue index" A high test-retest reliability for the fatigue test has earlier been demonstrated (Thorstensson 1976) A coefficient of variation of 3 2% was obtained based on day-to-day-measurements In order to evaluate whether subjects in the present study did exert MPT in the fatigue test initial peak values from a fatigue test were compared with MPT values as well as initial peak values obtained in a repeated fatigue test some days later The c v corresponded to 3 7 and 3 9% (n 22) respectively In addition 10 subjects were re-tested after 18 months During the time elapsed physical activity was maintained at an almost constant level comparable to the pre-test level. The reproducibility of the fatigue test expressed as c v was 6 9% while the corresponding value found for MPT was 5 3%

Maximal oxygen uptake (I II IV VI)

Maximal oxygen uptake was determined using leg exercise on a Monark cycle ergometer by stepwise increasing the work load according to the levelling off criterion (Astrand & Saltin 1961) Expired air was collected in Douglas bags and the volume measured in a spirometer For O_2 and CO_2 analyses a mass spectrometer (Centronic 200 MGA, Centronic Works Croydon England) was used Duplicate determinations revealed a c v of 1 2% (n 20)

Cycling exercise (VI)

To make comparisons possible with previous muscle fatigue studies short term maximal cycle exercise was applied This took place without any warm up period An exhaustive cycling test was performed on a Monark cycle ergometer with a work load corresponding to 120 (range 118-121) % of $\dot{V}O_2$ max Inability to maintain a prescribed pedalling frequency (i e. 70 rev x min^{-1}) was taken as criterion for exhaustion. The day-to-day variation in exercise tests of this kind (n = 10) was 3 1% (c v)

Downhill skiing (VII)

To study the effect of short term heavy muscle exercise in sport events subjects performed submaximal and maximal skiing of approximately 1 min duration on a slope adjusted for competition.

The glycogen depletion pattern (Gollnick et al. 1972a) was studied during two consecutive days of skiing in order to evaluate muscle fibre recruitment pattern. The exercise consisted of ski-school activity (physical-education students) and competitive slalom and giant slalom training (elite skiers) Training sessions for both groups lasted approximately 5 h per day

EMG recordings (III-IV)

To evaluate changes in electromyographic activity during the muscle fatigue experiments EMG was registered from the vastus lateralis muscle Surface electrodes (Hellige Stockholm) of 10 mm in diameter were placed over the belly of the muscle as close as possible to the site of the muscle biopsy insertion Muscle biopsies (see later) were taken at least one week prior to EMG experiments The interelectrode distance was 10 mm in the direction of the patella-crista iliaca.

Amplified EMG signals were either rectified and filtered by a single lag filter (120 ms time constant) and displayed on a UV Honeywell 2012 recorder along with the corresponding torque curve or amplified and immediately stored on magnetic tape (Philips Analog 7) with a recording speed of 380 mm x s^{-1} for further analysis Peak EMG was defined as the highest point on the rectified and filtered EMG curve while integrated EMG (IEMG) was taken as the area under the rectified and filtered EMG curve as measured by planimetry Analysis was limited to the mid-range of knee extension.

Using a laboratory computer (HP 2116 C) and a data processing system (Komi & Lehtiö 1973 Viitasalo & Komi 1975) IEMG and power spectral density function (PSDF) were obtained according to Bendat & Piersol (1971) In order to demonstrate changes in PSDF mean power frequency (MPF) was computed and the relative proportions were calculated for selected bandwidths (24-48 Hz 56-88 Hz 96-128 Hz and 136-400 Hz) For further description see Viitasalo & Komi (1977)

Muscle biopsy sampling (I-VII)

Muscle tissue specimens (approximately 30-50 mg) were taken from vastus lateralis using the percutaneous needle biopsy technique (Bergström 1962) This part of the quadriceps muscle has been found to be heavily activated when performing knee extensions during isokinetic conditions (Merrifield & Dostal 1978) maximal cycling exercise (Bigland-Ritchie & Woods 1974 Henriksson & Bonde-Petersen 1974) and down-hill skiing (Eriksson et al 1976)

Biopsies for histochemistry were mounted in embedding medium (Cryoform™ Damon, Needham Hts Mass) and frozen in isopentane cooled with liquid nitrogen. For biochemistry muscle biopsies were frozen directly in liquid nitrogen Samples were stored at -80°C until analysed. Specimens for glycogen determinations histochemical as well as biochemical were obtained within 5 min before and after exercise Muscle biopsies for lactate assays were obtained within 3-5 s after exercise with the subjects in a sitting position while muscle biopsies after skiing were taken within 10-15 s in a supine position.

Histochemistry (I-VII)

Serial transverse sections (10 µm) were cut in a microtome at -25°C Stainings were undertaken for ATPase activity (Gomori 1941 Padykula & Herman 1955) after preincubation at pH 10 3 4 6 and 4 3 respectively (Engel 1962 Brooke & Kaiser 1970) as well as for NADH-tetrazolium reductase (Novikoff et al 1961) For classification fibres were

identified as fast twitch (FT) and slow twitch (ST) fibres (Engel 1962) and for further subgroup classification of FT fibres (Brooke & Kaiser 1970) into FTa and FTb according to the terminology proposed by Saltin et al (1977) The percentage of each fibre type was calculated from sections containing at least 200 fibres but mostly comprising 400–500 fibres Muscle fibre area was determined on NADH-tetrazolium reductase stained fibres using a cutting and weighing procedure (Thorstensson 1976) At least 10 fibres of each fibre type subjectively rated as representative for the entire transverse sections were selected for analysis The relative muscle area occupied by FT muscle fibres was calculated according to the formula: (100 x FT/ST area x % FT) x (FT/ST area x % FT + % ST)$^{-1}$ Error of methods (c v) estimated from analysis of duplicate biopsies ranged 6–11% for area and distribution variables (Table 2)

Table 2 Methodological errors (c v) of histochemical and biochemical variables Samples used for lactate assay ranged 10–35 in concentration (mmoles x kg^{-1} wet muscle) Analyses were conducted on pools of fibres ranging 10–30 µg in dry weight Mean values were calculated from 2–3 pools of fibres and therefore each value represents a dry weight corresponding to approximately 40–80 µg

		c v %	n[2]
HISTOCHEMISTRY			
Muscle fibre type distribution[1]		6 2	28
Muscle fibre area[1]		10 5	30
Muscle fibre area ratio[1]		7 5	26
LACTATE			
ANALYSES UNDERTAKEN SIMULTANEOUSLY			
Pool to pool	unidentified fibres	6 8	35
—	FT fibres	8 7	35
—	ST fibres	7 2	35
—	divided fibres	3 6	12
Mean to mean	unidentified fibres	4 4	32
—	FT fibres	4 4	12
—	ST fibres	4 4	12
ANALYSES UNDERTAKEN ON TWO SUCCESSIVE DAYS			
Pool to pool	divided fibres	6 7	14
Mean to mean,	—	2 2	6
ANALYSES UNDERTAKEN ON DIFFERENT OCCASIONS			
Mean to mean		5 6	12

1) Duplicate biopsies

2) Duplicate determinations

To demonstrate selective glycogen depletion pattern cross-sections (16 µm) were PAS (periodic acid-Schiff) stained for glycogen (Pearse 1961) Individual fibres were subjectively rated as filled moderately emptied, and exhausted using a system similar to that proposed by Gollnick et al (1972a)

Biochemistry (I II VI VII)

Metabolites were determined in mixed muscle samples as well as in separated dissected out fibres After weighing at $-25^{o}C$ on a Cahn electrobalance muscle biopsies (approximately 10-50 mg) were analysed for their glycogen and lactate contents by means of fluorometer technique according to Lowry & Passonneau (1972) as modified by Karlsson (1971) using a Farrand ratio fluorometer model 24 (Farrand Optical Co New York)

Analyses on separate muscle fibres were performed in the following way: after lyophilizing muscle samples (approximately 2-12 mg) were placed under a microscope and 100-200 fragments of muscle fibres were dissected out from each sample at a constant room temperature and humidity ($20^{o}C$ and 30% respectively) Thereafter a part of each fibre was cut off and stained for fibre type identification (Essén et al 1975a) For lactate analysis 5-13 FT and ST fibre fragments respectively were pooled and weighed on a Cahn electrobalance or Quartz fibre ultramicro balance (Bodder "E" Microtech Services Berkely) Weight of the samples corresponded to 10-30 µg Fibres were then put into test tubes before the reagent solution was added. The lactate concentration was determined according to Karlsson (1971) Each value was calculated as a mean value for the lactate concentration in 2-7 pools Comparing values obtained from conventional analysis on whole wet (Karlsson 1971) or freeze-dried (Essén 1978) samples with the present method no systematic differences were noted in terms of lactate concentration although wet sample values tended to be lower thus confirming Karlsson (1971) Other methodological errors for lactate analysis estimated from duplicate determinations are listed in Table 2 All values were converted into wet weight values assuming a water content of 77% for muscle biopsy samples (Karlsson 1971) This value for muscle biopsy water content was confirmed and no differences were obtained irrespective of whether biopsies (n = 25) were taken at rest or after short term (60 s) heavy exercise However small intra- and interindividual variations in water content were present corresponding to 75-78%

In order to express lactate concentration for the entire muscle investigated, the following formula was applied: Lactate concentration in FT fibres x % FT area lactate concentration in ST fibres x % ST area Lactate dehydrogenase (LDH) activity and muscle specific LDH (M-LDH) activity for the entire muscle were calculated in a manner corresponding to lactate

For muscle tissue enzyme studies additional dissected pools of FT and ST fibres each consisting of approximately 100 fibre fragments were homogenized in 100 µl of 0 5 M KCl Total lactate dehydrogenase (LDH) (E C 1 1 1 27) activity in the forward reaction (pyruvate ⟶ lactate LDH_{tot}) was determined fluorometrically

(Lowry & Passonneau 1972) in the homogenate diluted 1:4 with 0 1 M Tris-HCl buffer pH 7 5 Discelectrophoresis for separating the LDH isozymes was performed according to Dietz & Lubrano (1967) on 25 µl of the homogenate The relative contribution of muscle specific LDH monomeres (% M-LDH) was determined using a densitometric scanning technique on the separated and stained LDH isozyme bands in the gels (Sjödin 1976) The activity corresponding to the muscle specific LDH (M-LDH) was then calculated (% M-LDH x LDH_{tot}) The error of method (c v) estimated from duplicate biopsies was 3 1% (n 10) (Sjödin personal communication)

Blood analysis

Blood samples were obtained from the finger tip and analysed for lactate concentration according to the Barker and Summerson method as modified by Ström (1949)

RESULTS AND COMMENTS

Muscle fibre type distribution in vastus lateralis of men (I-VII)

Muscle fibre type distribution in the vastus lateralis muscle in 64 healthy young men averaged 49 (range 10-79) % FT fibres The relative area occupied by FT fibres averaged 52 (range 10-77) % When subjects involved in competitive physical training were excluded, the values corresponded to 53 (range 29-79) % FT and 57 (range 28-77) % FT area respectively Individual FT/ST muscle fibre area averaged in the whole material 1 20 (range 0 70-1 85) and 1 24 (range 0 88-1 85) when trained subjects were excluded (Table 3)

Table 3 Mean (range) values for muscle fibre type distribution and area in the sample studied including (n = 64) and excluding 14 individuals involved in competitive physical sports

	Fibre type distribution % FT	FT fibre area 100 µm²	ST fibre area 100 µm²	FT area %	FT/ST area
n = 64	49 (10-79)	64 5 (35 9-101 7)	54 4 (29 4-88 9)	52 (10-77)	1 20 (0 70-1 85)
n = 50	53 (29-79)	66 8 (36 9-101 7)	55 5 (29 4-88 9)	57 (28-77)	1 24 (0 88-1 85)

Subgroup classification of FT muscle fibres was undertaken on samples from 23 subjects The mean distribution of FTa and FTb fibres was 30 (range 13–49) % and 19 (range 0–37) % respectively

Comments: The subjects were recruited among physical education students or individuals with approximately an equivalent physical activity level Fourteen athletes involved in competitive physical training were also included.

The results concerning fibre type distribution and fibre area in m. vastus lateralis of men revealed a pattern in agreement with previous studies (Edström & Ekblom 1972 Gollnick et al 1972b) Although there is a wide range in the distribution muscles of physically active though not competitive men seem to be composed of a smaller proportion of FT fibres as compared to muscles of individuals in the sedentary state as also reported by e g Gollnick et al (1972b) and Larsson (1978) Furthermore it was noted that individuals successful in endurance events displayed a more homogenous fibre type distribution characterized by a high percentage of ST fibres and a low occurrence of FTb fibres thus confirming other authors (Jansson 1975 Nygaard Jensen 1976 Jansson & Kaijser 1977 Jansson et al 1978)

Muscle strength and its relation to muscle fibre type distribution (I-V)

Peak torque attained at a relatively high speed of movement ($180^{\circ} \times s^{-1}$) either expressed per kg body weight or in absolute terms was positively correlated to the percentage of FT fibres as well as to the relative proportion of FT fibres (r 0 48–0 65 $p < 0\ 001$ n = 50) In addition peak torque was related to the absolute FT fibre area (r 0 30–0 33 $p < 0\ 05$)

In a subgroup (n = 18) peak torque at a higher angular velocity ($300^{\circ} \times s^{-1}$) was studied This speed is approximately 50% of the maximum angular velocity that could be obtained during knee extension (Thorstensson 1976) Peak torque averaged 131 Nm or 1 8 Nm x kg^{-1} b w as compared to 151 Nm or 2 1 Nm x kg^{-1} b w at $180^{\circ} \times s^{-1}$ The observed relation between the proportion of FT fibres and peak torque developed was not further strengthened by increasing angular velocity Approximately the same correlation coefficients were found when relating peak torque to percentage of FT fibres as well as to the relative area occupied by FT fibres No relationship could be demonstrated between peak torque and the relative occurrence of one or the other type of FT fibre subgroups

Comments: Peak torque developed at movements of relatively high angular velocities ($180\text{–}300^{\circ} \times s^{-1}$) was found to be related to the percentage of FT fibres thus confirming Thorstensson & Karlsson (1976) Since peak torque also was related to the absolute FT fibre area but not to mean fibre area there are reasons to believe that the quality of the activated muscle i e the proportion of FT fibres is of primary importance for force development at higher angular velocities This suggestion is in accordance with recent observations by Coyle et al (1979) Consequently the quantity i e the muscle mass as such is suggested to be of secondary importance in terms of

force development at high angular velocities Even though it has been possible to correlate mean fibre area with the cross-section of the muscle by means of dator tomography (Nygaard et al 1978a Häggmark et al 1978) such an interpretation must however be taken with caution

Muscle power and fatigue and the relation to fibre type distribution (II-VI)

Decrease in peak torque was found to occur within 10 maximal muscle contractions ($p < 0\ 001$ n 50) Muscle fatigue either expressed as the relative or absolute decline in peak torque after 50 contractions was positively correlated to the muscle fibre type distribution expressed as % FT ($r = 0\ 43-0\ 61$ $p < 0\ 01-0\ 001$) or % FT area ($r = 0\ 40-0\ 58$ $p < 0\ 01-0\ 001$) ($n = 50$) The relationship observed between fibre type distribution and relative decline in peak torque confirms the findings by Thorstensson (1976) In a subsample muscle fatigue was found to be related also to the occurrence of FTb muscle fibres (Fig 1) Approximately the same correlation coefficients were present when relating the decline in peak torque either expressing the fibre type population as above (r 0 53-0 57 $p < 0\ 01$) or to the percentage of FTb fibres ($r = 0\ 55-0\ 57$ $p < 0\ 01$)

Changes in peak torque were as expected accompanied by corresponding changes in power and work. Hence decline in power and work was correlated to the percentage of FT fibres ($r = 0\ 64-0\ 75$ $p < 0\ 05-0\ 01$ $n = 12$) The amount of work produced during the first 15 contractions (20 s) was approximately 20% greater ($p < 0\ 05$) in a group of subjects (n 5) with muscles made up by a high proportion of FT fibres (mean $\pm$ SE 62-4%) as compared to individuals (n 5) with a low proportion of FT fibres (37$\pm$3%) With additional contractions this difference became smaller and smaller After 100 contractions the cumulated amount of work was approximately equal comparing the two groups (Fig 2)

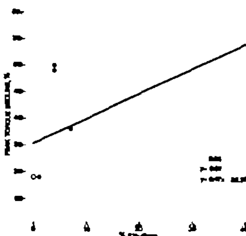


Fig 1 The relationship between the pe - centage f FTb muscle fibre in the vastus l t li muscl and the de line in peak torque with 50 maximal knee extensions t an angular velocity f 180° x $^{-1}$ (r ad x s^{-1}) (n 23) Individual with mus l fibre type di t ibution less than 33% FT fibres ar denoted with open (0) dots

LDH and its significance for lactate formation (I II VI unpublished results)

Correlation coefficients between LDH_{tot} as well as M-LDH activity and individual muscle fibre type distribution (% FT) were 0 96 ($p < 0\ 001$) and 0 98 ($p < 0\ 001$) respectively In addition the relative contribution of M-LDH in FT and ST fibres respectively was related to the percentage of FT fibres in the muscles investigated (r 0 74 $p < 0\ 01$ and r = 0 89 $p < 0\ 001$) i e the proportion of FT fibres in the muscle reflected the quality of the ST fibre in respect to its LDH activity

To study further the significance of LDH enzyme activity on lactate formation capacity 10 subjects performed 25 repeated muscle contractions according to Thorstensson (1976) Mean values were 18 5 (range 3 5–31 1) mmoles x kg^{-1} wet weight (lactate) 0 90 (range 0 38–1 65) (LDH_{tot}) and 0 60 (range 0 18–1 54 (M-LDH) mmoles x kg^{-1} x min^{-1} x 10^4 wet weight The individual differences in muscle lactate accumulation after exercise were found to be related to individual variations in LDH_{tot} (r = 0 66 $p < 0\ 05$) and M-LDH (r = 0 70 $p < 0\ 05$) activity This relationship was also present in 7 endurance trained individuals At exhaustion following a short term cycling exercise the individual variation in lactate concentration (mean and range were 20 8 and 13 9–30 5 mmoles x kg^{-1} wet weight) was found to be positively correlated to the percentage M-LDH (r 0 73 $p < 0\ 05$) (Table 4)

Comments: The data confirm earlier findings by Karlsson et al (1974) and Sjödin (1976) that muscle fibre type distribution will determine the metabolic quality of the muscle in terms of its potentials for lactate formation In addition to this an interaction appears to be present between the two muscle fibre types Thus in ST fibres of muscles rich in FT fibres the LDH activity as well as LDH isozyme pattern approached the properties of FT fibres more than in a muscle rich in ST fibres Whether or not this is an effect of adaptive mechanisms due to the environment remains speculative

Table 4 The interrelationship between some physiological histochemical biochemical and functional parameters obtained in 5 individuals performing short term high intensity exercise

Subject	$\dot{V}O_2$ max ml x kg^{-1} x min^{-1}	FT %	M-LDH %	Lactate concentration mmoles x kg^{-1} wet muscle FT	ST	whole muscle	Performance time s
I	70 0	29	39	14 4	13 6	13 8	99
II	65 9	29	45	26 2	15 5	18 8	101
III	66 6	40	39	26 7	21 4	23 8	119
IV	60 6	42	56	26 0	23 5	24 6	124
V	61 0	62	74	31 8	26 5	30 5	126

Taken together the data from the isokinetic one leg and cycling experiments seem to indicate that the activity and quality of the LDH-enzyme are critical for lactate formation and performance capacity Previously it has been demonstrated that physiological concentrations of lactate in vitro will decrease LDH activity (Karlsson et al 1974) Furthermore it has been shown that phosphofructokinase (PFK) activity considered to be the rate limiting enzyme for glycolysis is inhibited at low pH (Danforth 1965) A decrease in pH has been demonstrated concomitant to lactate formation in voluntary contracting human muscle (Sahlin 1978)

Myoelectric activity during fatiguing conditions (III IV)

Although a pronounced decrease in peak torque was demonstrated after 50 and 100 contractions corresponding to 50-75% peak EMG and IEMG decreased only slightly (n.s) in the whole material studied (n = 23) In a subsample of individuals (n = 6) with muscles with a high proportion of FT fibres IEMG decreased significantly (p < 0 01) As a consequence EMG activity for the whole material either expressed as peak EMG per unit peak torque or IEMG per unit work increased progressively to level off around the 75th contraction. The change (increase) in these ratios with 50 contractions was positively correlated to individual muscle fibre type distribution expressed as per cent FT fibres (r = 0 84 p < 0 001 and r = 0 78 p < 0 01) Consequently the increase in the IEMG/torque ratio was positively related to the relative decrease in peak torque (r = 0 88 p < 0 001) following 100 contractions

Different qualitative EMG changes were observed during repeated muscle contractions Thus a 25% decrease (p < 0 001) in power spectral density function (PSDF) of the EMG expressed as mean power frequency (MPF) was present in a group of individuals (n 6) with muscles rich in FT fibres (mean $\pm$ SE relative FT fibre area 67$\pm$3%) In a group of subjects (n = 5) with muscles not as rich in FT fibres (43-1%) a smaller nonsignificant decrease of MPF was present Intergroup deviations were noted already after 25-30 contractions (p < 0 01) When these two groups were combined individual relative change in MPF after 25 contractions was positively related to peak torque decline (r 0 74 p < 0 01) as well as to muscle fibre type distribution expressed as per cent FT fibres (r 0 60 p < 0 05)

Comparing the frequency spectrum changes between the two groups certain differences were observed. Increase in the relative content of the lower bandwidth (24-48 Hz) as well as decrease in the higher bandwidth (136-400 Hz) were significantly greater (p < 0 01 and p < 0 05 respectively) in subjects characterised by muscles rich in FT fibres

In addition to qualitative changes in EMG activity events linked to the neuromotoric control were found to be altered with a concomitant reduction in muscle force Thus individual differences related to muscle fibre type distribution were noted when studying the time lag between the first action potential of the EMG and the corresponding torque recording at a pre-set angular velocity ($180^0 \times s^{-1}$) (n 12) Values demonstrated initially and after 50 and 100 contractions were (mean $\pm$ SE)

95±3 115±5 and 121±5 ms respectively The time lag was initally negatively related to the percentage of FT fibres (r = −0 68 p < 0 05) and the percentage time lag increase after 100 contractions was positively related to % FT (r 0 73 p < 0 01)

Comments: The fatigue associated shift in EMG frequency spectrum towards low frequency components is in accordance with Kogi & Hakamada (1962) A significant drop in EMG activity as well as a more pronounced shift in the frequency spectrum of EMG with fatigue was noted in muscles with a high percentage of FT fibres as compared to muscles with a high percentage of ST fibres Although information regarding lactate concentration was not available from these experiments it can be assumed that after 25 contractions higher values were present in those subjects who demonstrated the most pronounced shifts in PSDF This reasoning seems to hold true since data obtained on individuals also taking part in experiments where lactate concentration was determined revealed an inter-relationship between force decline lactate accumulation (and LDH activity) and PSDF shift (Fig 5) Whether changes in time lag between onset of EMG and torque development at a pre-set angular velocity depend on biochemical processes taking place as a result of repeated muscle contractions cannot be established here Nevertheless the present data clearly indicate that the muscle rich in FT fibres initially has the shortest time lag but suffers more during repeated contractions as compared to the muscle rich in ST fibres

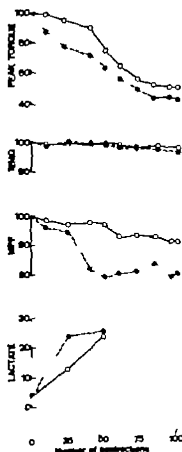


Fig 5 Changes (expressed as % of initial value) for two individuals in peak torque integrated EMG (IEMG) and mean power frequency (MPF) during repetition of 100 maximal knee extensions Muscle biopsies for lactate concentration (mmol x kg⁻¹ wet muscle) determinations were obtained in separate experiments The two subjects differed in respect to muscle fibre type distribution; subject GC (●): 54% FT fibres and subject GL (O) 34% FT fibres

Lactate formation during downhill skiing (VII)

In order to study the effects of acute physical activity in sports demanding great muscular power downhill skiing was used as an experimental model Low muscle glycogen values in the vastus lateralis muscle were noted after a day of skiing and in two samples studied levels below 15 mmoles glucose units x kg^{-1} wet weight were found Skilled and unskilled skiers demonstrated different selective glycogen depletion patterns In both groups a great loss of glycogen from the FT fibres was demonstrated However in the skilled skiers a pronounced depletion of ST fibres was observed as well Based on these findings it was suggested that downhill skiing to a high degree recruited the FT fibre population irrespective of training status This was supported by the fact that it was not possible to relate muscle lactate accumulation to level of proficiency However lactate concentration in the exercised muscle was positively correlated to the relative distribution of FT muscle fibres after maximal skiing (Fig 3) No such relationship was demonstrated following submaximal skiing conditions resulting in significantly lower lactate values Furthermore the FT/ST lactate ratio reached higher values during maximal skiing in individuals rich in FT fibres as compared to their opposites (r 0 84 $p < 0.05$)

Comments Low muscle glycogen levels were demonstrated after skiing and such values are only reported after prolonged intense running cycling or cross-country skiing Thus it can be concluded that downhill skiing competitive as well as recreational is performed with an enhanced utilization of glycogen especially in FT fibres and that the site of the quadriceps muscle chosen is representative to study muscle metabolism during downhill skiing

Downhill skiing is evidently a physical activity which is dependent on recruitment of FT fibres irrespective of training status and is therefore in some respects comparable to the different laboratory tests applied in the present study Accordingly it suggests that the FT fibres were more active in formation of lactate It can be suggested that downhill skiing especially in less skilled skiers will be characterized by a high degree of isometric muscle exercise This assumption is supported by the fact that a high FT/ST lactate ratio was observed in subjects rich in FT fibres which is in accordance with findings obtained during standardized isometric exercise at different tension levels (Tesch & Karlsson 1977)

The present data indicated that skilled skiers recruited relatively more of their ST fibre population than unskilled Nygaard et al. (1978b) observed pronounced glycogen depletion of FTb fibres in one inexperienced skier while none or minor loss of glycogen from these types of fibres was observed in more advanced skiers This provides further evidence of a contrasting recruitment pattern in skilled and unskilled individuals

GENERAL DISCUSSION

Metabolic profiles of muscle fibre types in man

Studies on separated human skeletal muscle fibre types disclose a consistent pattern regarding their metabolic profiles Thus in terms of metabolic potentials the ST fibre is suited for use in prolonged activities with reliance upon oxidative metabolism, indicated by higher activity of enzymes involved in the citric acid cycle e g succinate dehydrogenase (Essén et al 1975a Henriksson & Reitman 1976) as well as a LDH isozyme pattern favouring lactate oxidation (Sjödin 1976) Furthermore observations on habitually active men have indicated a greater blood flow in muscles rich in ST fibres as compared to muscles rich in FT fibres (Juhlin-Dannfelt et al 1979) Thus a relationship between leg vascular resistance and muscle fibre type distribution was demonstrated which fits well with the greater capillary density of muscles rich in ST fibres (Andersen 1975 Andersen & Henriksson 1977) Moreover a relationship between the mitochondrial content and number of capillaries surrounding each fibre has been demonstrated (Brodahl et al 1977) The FT fibre on the other hand, in addition to superiority in terms of contractile characteristics (Thorstensson et al 1977) display higher activity of enzymes thought to be rate limiting for glycolysis i e phosphorylase (Piehl & Karlsson 1977) phosphofructokinase (Essén et al 1975a Henriksson & Reitman 1976) and lactate dehydrogenase (Sjödin 1976 Thorstensson et al 1977) Even in terms of stored metabolites differences are present between the two main fibre types Slightly higher CP concentrations were noted in FT fibres while no differences in ATP concentration of fibre types were noted in the resting muscle (Essén 1978) Triglyceride content is significantly higher in ST fibres (Essén et al 1975a) whereas glycogen content seem to be approximately equal in different fibre types (Essén et al 1975a Piehl & Karlsson 1977)

In addition the subgroups of FT fibres are known to differ in terms of enzymatic and vascular profile In most respects the FTa fibre seems to represent an intermediate type in comparison to the ST and FTb fibre (cf Saltin et al 1977)

Muscle fibre type distribution as determined by staining for myofibrillar ATPase in the muscle reflects the acto-myosin ATPase activity which in turn is assumed to be correlated with intrinsic speed of muscle shortening (Bárány 1967 Bárány & Close 1971) in the investigated muscle Considering this the present results clearly demonstrate that individual fibre type distribution to a certain extent will be important for anaerobic performance capacity as indicated by repeated muscle contractions of high intensity

Muscle fibre recruitment

Established methods are available for evaluation of the muscle fibre recruitment pattern in vivo The degree of glycogen depletion in different fibre types by histochemical means is one method to evaluate whether a certain type of fibre has been metabolically active during exercise (Kugelberg & Edström 1968)

Gollnick and co-workers (Gollnick et al 1973a b 1974a b) found with this approach that ST fibres seem to be recruited at muscle contractions calling for less than 20% of maximal voluntary isometric contractions At tensions exceeding 20% FT muscle fibres are recruited in addition. In dynamic (cycling) exercise with an energy need below maximal oxygen uptake ($\dot{V}O_2$ max) ST muscle fibres are the first to loose glycogen. At supramaximal load corresponding to 120% of $\dot{V}O_2$ max muscle glycogen depletion pattern indicates an initial loss from FT fibres although there are reasons to believe that a major portion of the ST fibre population is recruited simultaneously The rate of pedalling (30–120 rev x min^{-1}) does not influence the glycogen depletion pattern. Moreover at intensities close to or above $\dot{V}O_2$ max both FT fibre types are recruited (Andersen & Sjøgaard 1976) However it is not possible to establish with this approach whether certain muscle fibre types are continuously involved during an entire pedal thrust or engaged only in short bursts Using the same experimental isokinetic exercise protocol as in the present study contraction velocity was found to influence muscle fibre recruitment pattern suggesting a greater involvement of FT fibres at faster velocities (Lesmes et al 1979)

Intramuscular substrates other than glycogen may have been utilized as indicated by Essén (1978) analysing single muscle fibres for their metabolic contents Furthermore a higher energy consumption per unit isometric tension developed has been demonstrated for FT as compared to ST fibres of hamster (Goldspink et al 1970) which may obscure the recruitment pattern as indicated by glycogen staining intensities In line with this is the finding that the rate of heat production in human skeletal muscles is 6 times higher in FT as compared to ST fibres (Bolstad & Ersland 1978) The rate of glycogen breakdown is also found to partly be dependent on individual muscle fibre type distribution (Juhlin-Dannfelt et al 1977) and consequently care must be taken when evaluating for fibre recruitment pattern using the glycogen depletion method.

In order to evaluate the orderly recruitment pattern of single motor units electromyography using fine needle electrodes (Hannerz 1974) and bipolar surface electrodes (Gydikov & Kosarov 1974) has been studied. Information on muscle fibre recruitment pattern in man has been obtained in isometric (cf Gydikov & Kosarov 1974 Grimby & Hannerz 1977) but also in isotonic (Grimby & Hannerz 1977) leg exercise The results confirm the size principle theory based on experiments on decerebrated cat experiments (Henneman et al. 1965) In addition, an initial selective recruitment of FT motor units was demonstrated during ballistic movements irrespective of muscle tension level (cf Grimby & Hannerz 1977) These results indicate that an exclusive pool of FT motor units always will fire at onset of a muscle contraction when muscle tension is rapidly increased and/or forceful FT fibres were also observed to be activated upon sudden and vigorous contractions in partially denervated human muscles (Warmolts & Engel 1972)

Muscle exercise and metabolism

The present findings indicate that it is possible to objectively detect a decrease in the individual s ability to produce maximum muscle force within 10 muscle contractions For such a short period of intense exercise no experimental data are available showing complete depletion of the immediate energy stores A certain reduction of muscle ATP and CP depots are however present after 10-15 s of heavy exercise (Hultman et al 1967 Bergström et al 1971 Saltin et al 1971)

The question may be raised whether or not a complete lack of ATP in the myofibrillar regions is possible Based on frog and rat experiments impairment of contractile function due to inadequate availability of myofibrillar ATP has been suggested by e g Fitts & Holloszy (1976 1977) Murphy (1966) stimulating frog sartorius muscles demonstrated a close relationship between tension output and the ATP content of a metabolically inhibited muscle However he concluded that it is unlikely that any ATP dependent limitations will be present in the mechanical response of muscles with intact ATP restitution mechanisms

Spande & Schottelius (1970) stimulated isolated mouse muscle to fatigue and observed a linear relationship between CP concentration in the muscle and tension developed in an isometric tetanus They proposed that CP depletion can be the underlying cause of fatigue Whether the contractile machinery was directly affected or not could not be established from their results

Information concerning the glycogen depletion pattern during brief exercise in the order of 10-25 contractions are not available but most probably muscle contractions irrespective of contraction velocity and tension output must be repeated for longer periods of time in order to exhaust certain fibres (Andersen & Sjøgaard 1976 Secher & Nygaard Jensen 1976 Essén 1978)

It is open to speculation whether exhaustion following supramaximal cycling exercise is partly attributed to substrate depletion of highly recruited FT fibres By repeating exhaustive supramaximal one minute bouts approximately 50% of the FTb fibre population was found to be exhausted of their glycogen content already after 5 bouts (Thomson et al 1979) Since this fibre type to a high extent seems to contribute to the force applied at each pedal thrust (see above) selective substrate depletion cannot be excluded as being a limiting factor in intense cycle exercises in the order of 1-2 min duration

Force generation and motor unit activity

In order to study the origin of fatigue changes in EMG activity and muscle force have been followed during various type of activity resulting in impairment of muscle function. During sustained constant submaximal isometric contraction increased EMG activity occurs (Merton 1954 Edwards & Lippold 1956 Lippold et al 1960 Kuroda et al 1970 Viitasalo & Komi 1977) This has been assumed to be due to recruitment of additional motor units and/or increased firing frequency to compensate for failure in contractility of already recruited fibres (Lippold et al 1960) In experiments

where the fatigue mechanism was studied in sustained maximal isometric contraction in man a fall in EMG activity concomitant to a loss in muscle force was observed (Stephens & Taylor 1972) The unchanged electrical activity to tension (E/T) ratio was taken as indicative of fatigue at the neuromuscular junction Ochs et al (1977) demonstrated also a drop in electrical activity when studying EMG and force relationship during rapid repetitive maximal isometric plantar flexion In comparison with Stephens & Taylor (1972) muscle tension was however found to decrease at a higher rate as compared to the EMG activity which implies an increased E/T ratio Hence muscle fatigue was indicated to be located peripheral to the neuromuscular junction

Dissimilarities regarding changes in E/T ratio during development of muscle fatigue may be influenced by factors such as type of contractions investigated (e g submaximal vs maximal isometric contraction isometric vs dynamic contraction slow vs fast dynamic contraction) It has also been suggested that migration of activity may occur within a muscle as well as from one muscle to another (Lippold 1955 Komi & Viitasalo 1976)

Studying human single motor units a decrease in firing frequency (Marsden et al 1971) has also been noted concomitantly with a fall in muscle tension (Gydikov & Kosarov 1974) indicating that EMG might fall without any neuromuscular block. Contradicting findings regarding E/T ratio and conclusions in the literature might be due to differences in type of motor units dominating in the investigated muscle By comparing the EMG activity of the human gastrocnemii and soleus known to differ in terms of histochemical properties (Gollnick et al. 1974c Edgerton et al 1975) Ochs et al (1977) observed a more pronounced increase in E/T ratio of the gastrocnemii muscle during repeated plantar flexions EMG studies of human dorsal interosseous muscles have shown that FT motor units fatigue more easily than ST units In addition a broader range in fatiguability was observed for FT motor units as compared to ST motor units (Stephens & Usherwood 1975) Decrease in the EMG activity during maximal effort is suggested to be due to inhibition of some motor units and/or changes in the form of motor unit potential e.g amplitude duration frequency spectrum. Support for the latter possibility was demonstrated in experiments leading to conditions referred to as ischaemic (Mortimer et al. 1970) In agreement with human experiments (Stålberg 1966) a decrease in the conduction velocity of stimulated cat muscle fibres was observed which was suggested to account for observed changes in the EMG frequency spectrum. The decrease in conduction velocity was more pronounced in the gastrocnemii as compared to the soleus muscle The authors suggested lactate as responsible for these changes although no data were obtained on lactate concentration

Lactate accumulation and muscle fatigue

One of the basic findings in the present study was the higher rate of lactate formation observed in FT as compared to ST fibres The results agree with the data obtained in animal experiments (Ogata 1960 Beatty et al 1963) and previously suggested by Fletcher (1914) A lactate flow" from FT to ST fibres may have occurred as discussed by Essén et al (1975b) mediated by membrane bound M-LDH as suggested by Sjödin (1976) and thus partly explaining the lactate content in ST fibres

Concomitant with a reduction in ATP and CP stores there is an increased lactate formation (Hultman et al 1967 Karlsson 1971) Lactate accumulation depends on lactate production and release As demonstrated lactate release in man is found to be maximal already at muscle lactate concentrations corresponding to approximately 5 mmoles x kg^{-1} wet muscle (Jorfeldt et al 1978) The authors did not indicate whether the assumed translocation hindrances for lactate were related to individual muscle fibre type distribution Following submaximal cycling exercise greater muscle/blood lactate gradients were observed in individuals with muscles rich in FT fibres as compared to what was found in individuals with muscles rich in ST fibres (Graham et al 1978) Accordingly the present results suggest that individuals with muscles rich in high oxidative (ST and/or FTa) fibres have a greater release most probably due to a more developed capillary network (Andersen 1975 Andersen & Henriksson 1977) This suggestion is in agreement with the findings by Folkow & Halicka (1968) comparing red and "white muscles of cat with respect to blood supply capillary surface area and oxygen uptake at rest and during exercise

A causal relationship between muscle fatigue and lactate accumulation has been suggested (Asmussen et al 1948 Karlsson 1971 Klausen et al 1972) Support for this view has been found in human experiments where performance capacity of longer duration (3-5 min) was found to be negatively affected by elevated blood and muscle lactate levels induced by previous exercise of other muscles (Karlsson et al 1975) Accordingly the same lactate concentrations were demonstrated during maximal exercise at various ambient pressures concomitant to increased performance time with increased ambient pressure (Linnarsson et al 1974)

In muscle biopsy specimens a close relationship between pH and lactate concentration immediately at termination of exercise has been demonstrated (Sahlin 1978) and the same muscle vein pH was demonstrated on exhaustion during maximal exercise at varying ambient pressures (Kaijser 1970) indicating that increased acidity in the muscle may determine the point of exhaustion The mechanism for lactate fatigue is however not clear A mechanism for high H concentrations to cause impaired muscle function has been demonstrated by Fuchs et al (1970) and Nakamura & Schwartz (1972) They showed that an increase in the H concentration decreases the number of calcium ions bound to troponin during the excitation-contraction coupling This would reduce the number of active actin-myosin interactions thus decreasing contractile force Experiments on skinned frog muscle fibres have supported this hypothesis (Robertson & Kerrick 1979) Thus intracellular acidosis depressed mechanical function at the

actomyosin interaction level In addition a lowered pH was observed to require a higher Ca^{2} concentration to result in a given level of activation Eberstein & Sandow (1963) have published evidence indicating that the functional site of fatigue is in the link that couples excitation to contraction either reducing the intensity of the excitation-contraction link or decreasing the sensitivity to activation Changes in flux of calcium were suggested to alter the operation of excitation-contraction link.

Recently Fitts & Holloszy (1976 1978) were able to demonstrate a linear relationship between twitch and tetanic tension and lactate concentration of stimulated frog sartorius muscle During 30 s of recovery however contractile twitch tension and ATP concentration increased rapidly while no significant reduction in lactate concentration occurred during this period In experiments where cat gastrocnemii and soleus muscles were stimulated to contract a discrepancy in fatigue pattern was demonstrated between muscles in accordance with Kugelberg & Edström (1968) Hammarberg & Kellerth (1975) A 10 s pause in stimulation was found to result in a complete recovery in contractile strength of the gastrocnemii muscle The soleus muscle on the other hand displayed a less pronounced recovery from fatigue A more rapid restitution of ATP due to higher activity of creatine phosphokinase (Burke & Edgerton 1975) in the fast contracting gastrocnemii muscle was suggested to explain in part the discrepancy in recovery pattern In preliminary experiments using the fatigue test described here a rapid restitution of contractile tension was observed during the first 15 s of recovery from exercise in spite of unchanged or slightly decreased lactate levels Due to limited number of subjects studied, the influence of fibre type distribution could not be elucidated.

Central versus peripheral fatigue

"Muscle fatigue is a complex phenomenon with numerous possible sites of origin. "Central fatigue in contrast to "peripheral fatigue was suggested already in 1903 by Setchenov and even though Bigland-Ritchie et al. (1978) calculated that central fatigue may account for 30% of total force loss in sustained isometric contractions of the human quadriceps muscle the phenomenon was not observed during the initial phase of contraction. Hence force produced through voluntary contractions and contractions stimulated via the femoral nerve decreased to a similar extent during the first 30 s of contraction. One cannot preclude that changes in the "milieu interne of the activated muscle via sensory receptors and their afferents will result in a central inhibition as has been suggested (e g Reid 1928 Lippold et al 1960 Asmussen & Mazin 1978a) When again considering experiments originally described by Setchenov (1903) Asmussen & Mazin (1978a b) it was demonstrated that exhausted individuals in a second bout of exercise were able to perform more work if diverting mental or physical activity were introduced in the pause instead of only rest The observation was considered to be due to a change in the balance between the outflow

of inhibitory and facilatory impulses to the central nervous system implicating that central inhibition might influence mechanical output during voluntary contraction

Whether or not central fatigue occurs failure at or distal to the neuromuscular junction must be considered mainly to limit the extent of voluntary contraction. Thus muscle contraction impairment may arise from failure of transmission at the neuromuscular junction action potentials which fail to propagate or from disturbances in calcium uptake release or binding capacity due to internal metabolic changes concomitantly affecting the coupling processes

SUMMARY

1 Physically active young men were studied in terms of muscle fibre type distribution in the vastus lateralis muscle and performance capacity during various types of short term exercise The percentage of fast twitch (FT) muscle fibres averaged 49 (range 10-79) % in the studied muscle

2 Muscle force (torque) was recorded during repeated isokinetic knee extensions The absolute as well as relative torque decline was taken as criterion for muscle fatigue and was found to be more pronounced in individuals with muscles rich in FT and/or FTb muscle fibres

3 Electromyography (EMG) was recorded from the vastus lateralis using surface electrodes It was analysed for integrated EMG (IEMG) and power spectral density function (PSDF) In contrast to IEMG a general decrease in mean power frequency (MPF) was observed during repeated muscle contractions This shift was due to a decrease in the high frequency bandwidth (associated with FT motor units) of EMG with a simultaneous increase in the bandwidth of low frequency components In individuals characterized by a high percentage of FT fibres MPF decreased to a higher extent as compared to individuals with a high percentage of ST fibres

4 Lactate concentration and the activity of LDH_{tot} and H-LDH were analysed in pools of different muscle fibre types After various types of exercise (25 isokinetic contractions supramaximal cycling exercise downhill skiing) lactate concentration was related to % FT fibres in the activated muscle Lactate concentration was also related to LDH_{tot} and M-LDH activity This seems to indicate a higher rate of lactate production in FT fibres as compared to ST fibres

5 After 25 maximal repeated contractions a relationship between force decline and FT/ST lactate ratio was demonstrated A similar relationship was also demonstrated for LDH_{tot} and M-LDH ratios indicating a close relationship between the metabolic profile of the different fibre types muscle metabolism and muscle function

6 Lactate accumulation and associated metabolic changes interfering with the contractile mechanism is suggested to be responsible for muscle fatigue in the situations described here.

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Preface

The cell is the working unit of living organisms and each cell depends on regulatory transport functions of its plasma membrane Lipids, arranged in a bimolecular leaflet, constitute 50% or more of the building material of the cell membrane, but the properties of a hydrophobic lipid membrane cannot account for the permeabilities of biological membranes to hydrophilic molecules and ions. This was recognized and expressed by *Danielli* even before functional properties of artificial lipid membranes had been studied "the membrane sandwich must contain active regions impermeable to most types of molecules through which selected ions or molecules can diffuse through the lipid barrier

Artificial membranes play an important role for the endeavours to understand the transport properties of cell membranes The general low permeability of unmodified lipid bilayers to ions and hydrophilic molecules was confirmed experimentally when Mueller & Rudin and Bangham showed that stable lipid bilayers—well suited for transport studies—can be formed *in vitro* It was soon found that many characteristic features of the selective permeability properties of biological membranes are mimicked by modified artificial lipid membranes. The use of chemical compounds, which can induce selective permeabilities in artificial membranes have thus become instrumental for understanding general physical and chemical principles of specialized transport processes. Identification and isolation of integral membrane proteins responsible for biological transport phenomena, have been followed by promising attempts to "reconstitute" transport systems in artificial membranes. There is also substantial evidence that model systems will prove valuable for the investigation of interactions between membranes and membrane active hormones, toxins and drugs

The present volume contains contributions from a symposium, Ion Transport across Lipid Bilayer Membranes organized jointly by the Faculty of Natural Science and the Faculty of Medicine May 30 1979 on occasion of the 500th anniversary of the University of Copenhagen The chairman of the symposium was Professor D C. Tosteson (Harvard Medical School Boston Mass U.S.A) who received an honorary degree as doctor of medicine at the anniversary celebrations in recognition of his important contributions to basic membrane research essential for the development of medical science.

The symposium was organized in close collaboration with Professor Tosteson We feel that the invited speakers succeeded in their difficult task of presenting reviews of the major achievements in the field of lipid membrane research to an audience of scientists working mainly with other aspects of biological transport processes It is the hope of the editors that the informative and stimulating presentations will reach a larger forum through this publication

The symposium was made possible through a generous grant from the NOVO-Foundation.

The Editors

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On bilayers and biological membranes

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It is now about 20 years since Mueller et al. (1963) introduced the technique of forming planar lipid bilayers between aqueous solutions. They suggested that this model system could be useful in exploring the physical chemistry of biological membranes. Scientists interested in the transport of ions, and particularly the electrical properties of biological membranes soon began to use the bilayer system During the past decade many reports of such investigations have appeared in the literature (for recent reviews see Andersen (1978) Hall (1978) and Stark (1978))

This paper is a brief and personal assessment of the significance of research on bilayers for our understanding of how ions move across biological membranes. I give special attention to two lines of work that have been pursued in our laboratories. One line is the role of the dipole potential in regulating the movements of ions across bilayers and biological membranes. The other line is the relation between the primary structure of peptides and depsipeptides and their capacity to bind and promote transport of ions.

Some contributions of research on bilayers to ideas about ion transport across biological membranes

In the 1950's when I began my work on the molecular mechanisms and cellular functions of ion transport across membranes ion exchanger membranes were often put forward as a useful model (for example see Tosteson, 1955) However their thickness and composition were very different from those of biological membranes. The advent of planar lipid bilayers particularly those containing little solvent (Montal & Mueller 1972), as well as closed lipid vesicles (Bangham, 197?) made available for the first time a model bearing considerable resemblance to the membranes present in living cells. One important impact of bilayer research then, has been to make concepts of ion transport in biology considerably more realistic For example measurements of the extremely low ion permeability of unmodified bilayers made of lipids extracted from biological membranes (Andreoli et al 1967 a), contributed to the emergence of the notion that ion movements through biological membranes are mediated by proteins which penetrate through an otherwise largely impermeable liquid lipid matrix. With the increasing availability of pure membrane lipids membrane-active compounds, and more recently membrane proteins, we are slowly step by step drawing a molecular picture of biological membranes and the ways that ions and other substances move through them.

Some of the most important ideas that have emerged in this process are illustrated by the program of this symposium For example definite proof of the occurrence of ion conducting channels in bilayers through investigation of the effects of EIM, gramicidin alamethicin and other compounds (see Hall 1978) have stimulated much interest among physiologists studying excitable membranes. These simple systems have permitted quantitative and precise characterization of ion selectivity kinetics of ion movement and voltage dependence of channel opening and closing. As is made clear in other papers in this volume (Andersen 1980, Läuger 1980 Latorre & Donovan 1980) one important lesson from these investigations is that even with very simple systems made with known chemical components many important questions about how ion conducting channels work remain unanswered. This experience provides a guide to the limits of useful speculation about ion transport through channels in biological membranes.

Another important use of bilayers in understanding biological membranes is in the reconstitution of biological transport systems. Because of the parallel arrangement of transport pathways in biological membranes, it is usually difficult or impossible to identify in the native membrane the class of mem-

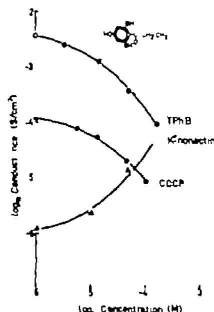


Fig 1 The effect of 2 4 6 trihydroxy propiophenone on the conductance induced by $TPhB^-$ (O) $CCCP^-$ (●), and nonactin (Δ) in phosphatidylethanolamine membranes. In the TPhB and $CCCP^-$ experiments the membranes were formed in 1 *M* NaCl, 10 m*M* PO_4, pH 6 TPhB was added to both sides of the membrane to concentration of 6.6×10 *M* $CCCP^-$ was added to both sides of the membrane to a concentration of 3.3×10^{-4} *M* In the nonactin experiment the membrane was formed in 1 *M* KCl 10 m*M* PO_4, pH 6. Nonactin was added to a concentration of 10^{-8} *M* Temperature 20 ± 1°C

...rane macromolecules responsible for a particular transport process. Isolation separation and reconstitution in a bilayer devoid of extraneous components is therefore the most reliable route toward molecular definition of a biological transport system In general vesicles have proved more useful than planar bilayers for reconstitution experiments (see for example Racker 1978) perhaps because the planar films used in most of the attempts at reconstitution have contained some hydrocarbon solvent Notable achievements in reconstitution of biological transport systems include the Na-K pump (Goldin 1977) the Ca pump from sarcoplasmic reticulum (Racker & Eytan 1975) and the proton pump from purple bacteria (Racker & Stoeckenius 1974) and the facilitated monovalent anion transport system found in mammalian red cells (Rothstein et al 1975) In general the work reported to date has established the feasibility of reconstitution in bilayers of transport systems isolated from biological membranes but has not yet contributed much to our understanding of how these molecular machines work In this regard some of the investigations reported elsewhere in this volume (Anner 1980) give hope for the future

Another promising direction of reconstitution experiments involves membrane receptors of hormones and drugs Both the identification of such receptors and the impact of their interaction with agonists and antagonists on membrane properties, can be explored in bilayers. An example of this line of investigation is also present elsewhere in this volume (M T Tosteson et al, 1980)

Many results of research in bilayers with important implications for thinking about ion movements in biological membranes could not be represented elsewhere in this volume The list includes the mechanism of action of uncouplers of oxidative phosphorylation (Liberman et al 1969 LeBlanc 1971) the significance for transport of chemical reactions involving transported substances in the relatively thick unstirred aqueous layers on the surface of membranes (Gutknecht et al 1977) the role of surface charge in modifying the ionic composition of the water immediately adjacent to membranes and thus the transport of these ions across the membranes (McLaughlin et al 1970) and the unequivocal demonstration of exchange diffusion in bilayers and a biological membrane (Wieth & Tosteson 1979 Tosteson & Wieth 1979) The remainder of this paper concerns two additional ideas emerging from investigations of bilayers

The role of the dipole potential in regulating ion transport across bilayers and biological membranes

Lipid bilayers are several orders of magnitude more permeable to lipid soluble anions than to lipid soluble cations of comparable size and shape (Liberman & Topaly 1969 LeBlanc 1970) Furthermore the surface potential produced at air water interfaces by monolayers of phospholipid is oriented in the direction that the hydrophobic air facing surface is positive relative to the underlying aqueous phase (Davies & Rideal 1963) These facts led Liberman & Topaly (1969) and LeBlanc (1970) to suggest that the electrostatic potential in the hydrophobic interior of bilayers is positive relative to the membrane surfaces This effect presumably arises from the interaction between the hydrophilic head groups of the membrane forming molecules particularly the carbonyl oxygen atoms in the ester

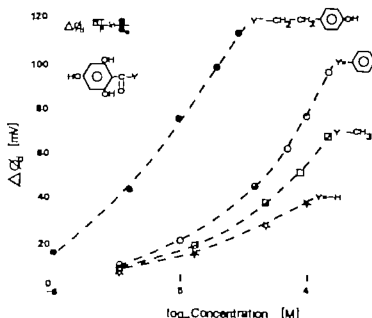


Fig 2 The change in dipole potential ($\Delta\phi_d$) promoted by different phloretin analogues. $\Delta\phi_d$ was calculated on the basis of the equation given in the upper left corner of the Figure. G is the conductance of the membrane at a given analogue concentration in the aqueous phases and G_o is the conductance of the unmodified membrane. Points are referred to TPhB⁻ conductance changes. Similar results are obtained with other ionic probes.

bonds of phospholipids with water dipoles in the adjacent aqueous solutions and hence has been called the dipole potential (Haydon & Myers 1973). Cass, Andersen and their colleagues (1973) and Andersen et al (1976) observed that phloretin the inhibitor of glucose and facilitated anion transport in red cells and other systems increases cation but decreases anion permeability in bilayers. They suggested that phloretin produces these effects by reducing that is making less positive the dipole potential. We have confirmed and extended their observations (Melnik et al 1977).

Cousin & Motais (1978) have recently observed that a series of phloretin analogues inhibit facilitated anion transport in red cells with differing potencies. They have attempted to rationalize their results in terms of the intrinsic dipole moment and hydrophobicity of the compounds. We have recently observed the effect of these compounds on the dipole potential in lipid bilayers (Latorre et al 1979). Table 1 shows the structures of the compounds that we have studied. The intrinsic dipole moment of the compounds was varied by altering the number and position of –OH residues on the aromatic ring. The hydrophobicity was varied by changing the substituents adjacent to the carbonyl group.

An example of the effect of one of these compounds, 2, 4, 6 trihydroxy propriophenone, on the conductance of bilayers to lipid soluble anions and cations is shown in Fig 1. The conductance of bilayers exposed to the lipid soluble anions tetraphenyl boron and CCCP⁻ decreased while the conductance of bilayers exposed to the lipid soluble cations complex K-nonactin increased as the concentration of the propriophenone increased. The magnitude of the change in the dipole potential of the membrane can be estimated from the change in conductance for anions and/or for cations. A graph of the change in dipole potential as a function of the concentration of phloretin and several of the analogues that we have studied is shown in Fig 2.

Using this approach we estimated the reduction in dipole potential produced by various concentrations of compounds shown in Table 1. From these data, we calculated the magnitude of the change in dipole potential ($\Delta\Delta\phi$ in millivolts (mV)) in bilayers produced by the concentration required

Table 1

X	Y	[illegible]		[illegible]	[illegible]
2,4,6 [illegible]		7	[illegible]		
2,4,6 [illegible]	-Cl	1.3	[illegible]	[illegible]	[illegible]
2,4,6 [illegible]	[illegible]	1	[illegible]	[illegible]	[illegible]
2,4,6 [illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
2, ,6 [illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
2,6 [illegible]	-Cl	[illegible]	[illegible]	[illegible]	[illegible]
2, [illegible]	-Cl	1.7	[illegible]	[illegible]	[illegible]
2, [illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
3 [illegible]	-Cl	[illegible]	[illegible]		[illegible]
7 [illegible]	[illegible]	3.7	[illegible]	[illegible]	[illegible]

OBTAINED FROM J COUSIN AND R MOTAIS (1978) B B ACTA [illegible]

to reduce the facilitated anion transport in beef red cells to one half of its maximum value (Cousin & Motais 1978) The results of these calculations are shown in Table 1 Note that the reduction in dipole potential was between 10 and 20 mV for most of the compounds studied

These results raise the question of a correlation between the action of phloretin and other acetophenone derivatives on the dipole potential in bilayers and their capacity to inhibit facilitated transport systems in biological membranes The relatively low specificity and potency of these compounds as inhibitors of biological membrane transport systems are consistent with the hypothesis that they exert their effect on some general property of membranes. From the expected reduction in positivity of the intra-membrane electrostatic potential it is reasonable to suspect that transport processes inhibited by these compounds share the property that the movement of negative charge across the membrane interior is rate-limiting. Another possible interpretation is that the large dipole potential difference is important in maintaining the shape and orientation of transport proteins in biological membranes Clearly these two lines of speculation are not mutually exclusive.

In the light of these results it is interesting to ask about the role of local electrostatic potentials in the mechanism of action of various membrane agonists and antagonists. Most neuro transmitters and membrane active hormones have significant permanent or induced dipole moments and many possess a formal electrical charge Perhaps one role of membrane receptors is to fix and thus increase the local concentration of an agent with the capability of altering the local electrostatic fields and thus the position and mobility of ligands on nearby membrane proteins

The relation between molecular structure and transport function of cyclic peptides and depsipeptides

Valinomycin has long been known to react with monovalent cations to produce positively charged complexes which readily traverse biological membranes (Pressman 1965) and lipid bilayers (Mueller & Rudin 1967 Lev & Buzhinsky 1967 Andreoli et al 1967b) Relatively minor changes in the primary structure of this cyclic dodecadepsipeptide profoundly alter its capacity to act as an ion carrier in membranes (Shemyakin et al 1965) Many substitutions of single residues interfere with the formation of the six intra-molecular hydrogen bonds that stabilize the bracelet-shaped complex between valinomycin and cation However certain analogues retain the capacity to form stable ion complexes and yet exhibit striking differences from the parent compound in their ability to promote ion transport across membranes. We have studied one such series of compounds that produced by substituting D or L proline for D isohydroxyvalerate and L lactate respectively (Gisin & Merrifield 1972 Ting-Beall et al 1974 Davis et al 1976, Benz et al 1976, Gisin et al. 1978) The primary structures of these compounds are.

Valinomycin (val) cyclo (Dval–Llac–Lval–Dhyv)$_3$
PV lac cyclo (Dval–Llac–Lval–Dpro)$_3$
PV cyclo (Dval–Lpro–Lval–Dpro)$_3$

These rather similar compounds differ markedly in their capacity to promote transport of ions across bilayers. For example the maximum current produced by a high electrical potential difference across glycerolmonooleate bilayers exposed to 10^{-6} *M* cyclic peptide and 0 1 *M* KCl are about 3×10^{-8} 3×10^{-6} and 10 A cm for PV PVlac and val respectively Thus replacement of the three hydroxyvalerate residues in valinomycin by proline reduces the maximum current by a factor of 10^{4} and the additional substitution of prolines for the lactate residues produces a further large reduction Furthermore unilateral addition of cyclic pep-

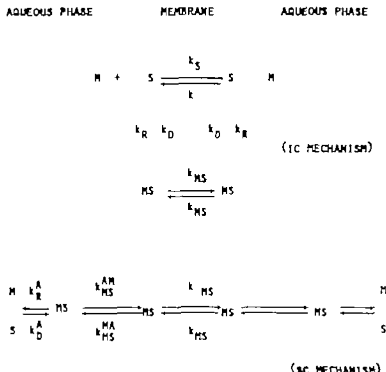


Fig. 3. Reaction scheme for the transport of an ion M by the carrier S. In the interfacial complexation mechanism (IC), the comple MS⁺ is formed at the membrane solution interface. In the solution complexation (SC) mechanism, the complex is formed in the aqueous phases.

tide to bilayers separating otherwise identical salt solutions (e.g. 0.1 *M* KCl) produces a steady state electrical potential difference in the case of PV but not for PVlac and val (Ting-Beall et al. 1974; Latorre & Koroshetz, unpublished data). Observations about the effect of these compounds on both the steady state and the kinetic characteristics of ion transport across bilayers can be interpreted in terms of the relatively simple model shown in Fig. 3.

The ion carrier S (one of the three cyclic peptides) can react with a metal ion M to form a positively charged complex, M S. This reaction can occur either in the aqueous solutions or as an interfacial reaction between M in the aqueous solution and S on the membrane surface. Both M S and S can adsorb to the membrane as defined by the partition coefficients β_{ms} and β_s and translocate between the surfaces at rates determined by the differences in interfacial concentration and values of the rate of coefficients k_{ms} and k_s. Because the complex M S is positively charged its movement is also affected by electrical potential differences between the membrane surfaces. Two general classes of transport mechanism can be distinguished. In one type interfacial complexation the transported ion reacts with the cyclic peptide on the membrane surface. In the other type the transported ion reacts with the carrier in the aqueous solutions and moves through the unstirred layer as the complex which then adsorbs to and moves through the membrane. This solution complexation mechanism can occur only when the rate coefficients for the reaction k_R and k_D, and/or the rate of coefficient for translocation of the free carrier k_s are too slow to permit the cycle necessary for the interfacial complexation mechanism to proceed sufficiently rapidly.

Values for the rate coefficients for translocation k_{ms} and k_s the rate coefficients for the interfacial reaction k_R and k_D, the partition coefficients β_{ms} and β_s and the aqueous dissociation constant k_{s}, for the three compounds in glycerolmonooleate membranes are shown in Table _. As shown clearly and convincingly by Läuger and his colleagues (Stark et al. 1971) valinomycin operates as an ion carrier by the interfacial complexation mechanism and all four

Table 2. *Rate constants for valinomycin-mediated and valinomycin analogs mediated K transport*

	k_R (M^{-1} s^{-1})	k_D (s^{-1})	k_{MS} (s^{-1})	k_S (s^{-1})	β_S (cm)	β_{MS} (cm)	K (M^{-1})	REF
val	3.0×10^5	3×10^5	2×10^5	4.0×10^4	0.004	–	<0.1	*b c*
PV Lac	2.3×10^5	4×10^5	5×10^4	5.5×10^1	0.0023	~0.007	≤1.5	*d*
PV	–		5×10^3	–	–	0.003	8	*e*

Glycerolmonooleate membranes Stark & Benz (1971) Benz & Lauger (1976) Present results. Benz et al. (1976)

of the relevant rate coefficients have approximately the same value of 10^5 sec^{-1} PV lac and PV function less well as ion carriers not because they fail to partition into the membrane or bind cations but rather because of their kinetic properties In fact, the partition coefficient for the free form of PVlac β_S is not significantly different from that for val while both PV lac and PV have a much greater affinity than val for K in water The greater affinity of PV for cations is even more evident in nonaqueous solvents (Davis et al 1976) The most striking difference between PVlac and val is a reduction in the value for the rate coefficient for translocation of the free peptide k_S by a factor of almost three orders of magnitude This value of k_S permits PV lac to carry K by the interfacial complexation mechanism but at a much slower rate in the steady than val For PV the values for k_S, k_R and k_D, were too low to estimate by electric relaxation techniques and this compound promotes K movement across bilayers by the solution complexation mechanism. Only k_{MS} the rate coefficient for translocation of the ion complex could be measured for all three compounds For PVlac it is four-fold and for PV forty-fold lower than for val

It is interesting to inquire about the molecular basis for the impressive differences between these three cyclic dodecapeptides as ion carriers. On the basis of their proton nmr spectra it is highly probable that the shape and size of the K complexes of val and PV are almost identical (David et al 1976) Therefore the forty-fold reduction in k_{MS} for PV compared with val is probably not due to differences in the mobility of the complexes within the membrane A more likely explanation is that the presence of the cyclic proline side chains rather than the isopropyl and methyl side chains of the hydroxy acids makes the hydrophobicity of the external surfaces of the PV K complex different from that of the val-K complex PVlac might be an intermediate case

Of even greater significance for their effectiveness as ion carriers are the differences in k_S the rate coefficient for translocation of the free form of PVlac and val Proton nmr experiments show clearly that the number of conformers of the free form of PV is greater than for val Furthermore the rates of the transitions between these forms is slower for PV and for val (Davis et al 1976 Grain et al 1978) This result is not surprising given the relative rigidity of the proline residues Indeed the slow rate of dissociation of the PV K complex revealed in the nmr data probably derives from the same property of proline and is in large part responsible for the fact that PV promotes K movement across bilayers by the solution rather than the interfacial complexation mechanism. Slow transitions between multiple conformational states of the free forms could explain the low values of k_S for the proline-containing compounds. The confomer released upon dissociation of the ion complex could be relatively hydrophilic and traverse the membrane only very slowly The slow conversion to a more hydrophobic confomer could limit the rate of transport.

The analysis of these three cyclic peptides illustrates that relatively minor and subtle changes in primary structure can have profound and complicated effects on their properties as ion carriers As we come to understand the primary structures of biological membrane transport proteins it is likely that similar complexities will become evident. Perhaps the lessons learned with these simple systems will be useful in addressing the problems of ion transport mediated by macromolecules.

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Reconstitution of the Na^+ K^+ transport system in artificial membranes

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ANNER, B M. Reconstitution of the Na K -transport system in artificial membranes. Acta Physiol Scand 1980, Suppl. 481 15–19 Department of Pharmacology Ecole de Médecine, Geneva, Switzerland.

(1) Purified renal N K -ATPase was incorporated into phosphatidylcholine-liposomes by using the cholate-dialysis procedure The passive permeability of the liposomes for Na and K was determined on ^{22}N and ^{86}Rb equilibration curves. The rate constant for passive Na-flux was 0.014 min^{-1} and for K(^{86}Rb)-flux 0 013 min Active transport rates of N and K were determined after addition of 3 mM ATP to the liposomes. (2) A micro-method for continuously measuring the ADP production associated with active transport is described. Using this technique initial velocities of ATP hydrolysis were measured in parallel with Na -transport and K -transport. When the Na -concentration in the incubation medium is increased, the initial velocity of all three processes increase concomitantly At high turnover the stoichiometry of coupled Na K -transport catalyzed by the purified reconstituted pump is 2.2N 1K 1ATP (3) When the pump has exhausted the vesicular K^+-pool, coupled Na K -transport ceases. The pump then carries Na uphill without K -antiport. The N $^+$ ATP ratio of uncoupled N -transport is close to 1 1

Key word Reconstituted Na K -pump stoichiometry uncoupled Na -transport

Most living cells expell sodium ions and accumulate potassium ions. This coupled transport process is mediated by an ATP-driven pump located in the cell membrane The stoichiometry of the Na K pump in red blood cells and in excitable tissues is around 3Na 2K 1ATP (for a review of the sodium pump see Glynn & Karlish 1975)

From early work on crude Na -K ATPase from peripheral nerve (Skou 1957) and from later work on the purified enzyme it became very probable that Na K ATPase was the Na K transport system (for recent reviews of the Na K ATPase see Schwartz et al 1975 Jorgensen 1975 Skou 1975) This was confirmed by the important demonstration that Na K ATPase reconstituted in liposomes catalyzed ATP-dependent Na -transport (Goldin & Tong 1974 Hilden et al. 1974 Racker & Fisher 1975)

There is much circumstantial evidence that the Na K -pump is also responsible for transepithelial Na -transport (Koefoed-Johnsen & Ussing 1958 DiBona & Mills 1979) The observation that Na K ATPase from dogfish rectal salt gland (Hilden & Hokin 1975) or from renal outer medulla (Anner et al 1976, 1977) catalyzes coupled active Na K transport after reconstitution in liposomes supports this concept. Moreover it has been observed that the Na -transport capacity of purified Na K ATP appears to be flexible to some extent (Anner et al 1977) This finding renders the role of the Na K -pump in transepithelial transport even more plausible

In the present work the nature of the additional Na transport component was investigated To that end, initial transport velocities Na K -gradients and the ATP hydrolysis associated with transport were measured. These combined methods have allowed to determine the coupling ratio of the

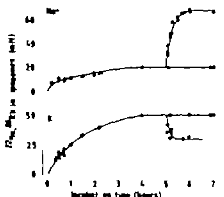


Fig 1 Time course (O) for loading of liposomes with ^{22}Na and ^{86}Rb Liposomes were formed in solution A containing 20 mM NaCl and 50 mM KCl and were incubated for 60 min at 25°C. At zero time on the abscissa, tracer was added and the isotope content of the liposomes was measured after removal of free isotope by gel filtration on Sephadex G-50 medium columns and calculated as a fraction of the vesicular isotope content at equilibrium. Active transport (●) was then initiated by adding 3 mM Tris-ATP to aliquots of the liposome suspension. The ATPase activity of free enzyme was inhibited with 1 mM ouabain when the active transport assay was started Net active cation transport was calculated from the difference of the radioactivity between liposomes incubated with ATP and control liposomes incubated without ATP

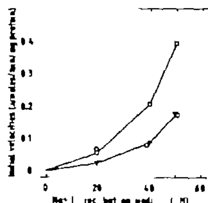


Fig 2 Initial velocities of Na -transport (□) K -transport (▽) and ATP-hydrolysis (O) as a function of the Na -concentration in the incubation medium. Liposomes were prepared in solution A containing 20 40 or 50 mM NaCl KCl was kept constant at 50 mM Initial velocities were measured between 30 and 300 s after ATP addition. The change of the isotope content was calculated as described in the Legend for Fig. 1 The initial velocity of transport-associated ATP-hydrolysis was read on recordings obtained as described under Methods.

Na K -exchange and to confirm that a fraction of e active net Na -transport is not coupled to K antiport

METHODS

Na K ATPase from the outer medulla of rabbit kidney was purified by the angle otor procedure described by Jørgensen (1974) Protein was determined according to Lowry et al (1951) in the presence of 0.1% sodium dodecylsulfate

For solubilization, 1 mg Na K ATPase protein with a specific activity of 800–1200 μmoles Pi/mg protein/h was sedimented at 120 000 g for 180 min and the pellet was resuspended in 900 μl of solution A choline chloride 50 mM L-cysteine 1 mM EDTA 1 mM $MgCl_2$ 5 mM varying concentrations of NaCl and KCl, imidazole 30 mM pH 7 10 and in addition 1% sodium cholate The undissolved enzyme was sedimented at 100 000 g for 15 min.

After addition of phosphatidylcholine to the soluble enzyme liposomes were formed by dialysis and active transport was measured as described before (Anner & Jørgensen 1979) except that 1 mM ouabain was present during the active transport assay

The transport associated ATP hydrolysis was measured by a modification of the linked-enzyme assay (Schwartz et al. 1971) and the time course was continuously recorded A 500 μl continuous flow quartz-cuvette was placed in a Unicam SP 1800 spectrophotometer through thin tubing with 490–495 μl of solution A containing in addition Tris-ATP 3 mM oubain 0 2 mM NADH 0 1 mM phosphoenolpyruvate 1.3 mM and 4 μl of a combined pyruvate kinase/lactatedehydrogenase suspension (Boehringer) The baseline was recorded for 20 min at 25°C. Then 5–10 μl of the liposomes suspension were added and the tubing was rapidly rinsed with a portion of the cuvette solution. The ATPase activity of free enzyme was negligible in the presence of 0.2 mM ouabain. The initial velocity of the ATP hydrolysis as well as the succeeding non linear part and the final linear part at steady state were recorded

RESULTS

Active and passive fluxes of Na and K in liposomes

For the study shown in Fig. 1 the liposomes were prepared in a solution containing 20 mM NaCl and 50 mM KCl The suspension was then incubated at 25°C and equilibrated with ^{22}Na or ^{86}Rb within 4 to 5 hours The rate constant for passive flux was 0 014 min for K and 0 013 min for Na

When ATP was added to the equilibrated liposomes the vesicular Na increased 2 9 fold whereas the vesicular K fell to 60% of the initial K content. Calculated on the total vesicle pool the Na -gradient was 48 mM and the K gradient 20 mM at steady state Although active Na K

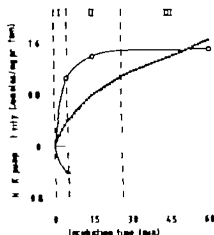


Fig. 3. Time course of active N -transport (O) K transport (∇) and ATP hydrolysis (■–■–■). Liposomes were prepared in solution A ontaining 40mM NaCl and 50 mM KCl. Net active cation transport was started by adding 3 mM Tris-ATP to the liposomes at isotopic equilibrium. Active cation transport was measured as explained in Fig. 1. The ATP hydrolysis curve was plotted from recording obtained as described under *Methods*. Phase I was delimited when K -transport reached a plateau and phase II when Na -transport reached plateau. Phase III designed N K -gradients at steady state

transport proceeded slowly when the incubation medium contained 20 mM Na and 50 mM K (See Fig. 2) the rate constant of active flux were 7–10 times larger than for passive Na and K fluxes. The pronounced difference of the passive and active Na K fluxes allows the precise determination of the initial velocity of active transport in phosphatidylcholine-liposomes

The stoichiometry of the ATP-dependent Na K -transport

The initial velocity of Na -transport K -transport and ATP-hydrolysis was determined at 20 40 and 50 mM Na while K was kept constant at 50 mM Fig. 3 illustrates that at 50 mM Na the initial velocity was 8 times larger for Na -transport, 7 times larger for K -transport and 3 5 times larger for ATP hydrolysis as compared to the velocities measured at 20 mM Na When the rates at 50 mM Na were compared to the rates obtained at 40 mM Na the increase was close to 2 for all three processes. Consequently the Na K coupling was rigid despite a 8 fold increase of the initial transport velocity The average coupling ratio was 2Na 1K

ATP hydrolysis was tightly coupled to active Na K -transport at 40 and 50 mM Na and the ratio was 2Na 1K 1ATP However at 20 mM Na where the transport velocity was low (Fig. 2) the number of ATP molecules hydrolyzed was equal or higher than the number of Na transported. This would imply that increasing Na in the incubation medium from 20 to 40 mM or 50 mM activates a Na site which not only stimulates initial transport velocities, but also facilitates the coupling between ATP hydrolysis and active Na K -transport. Yet, a more detailed analysis of this specific problem is necessary to consolidate this observation. The existence of multiple Na sites appears also from the initial sigmoidal relationship between transport velocity and Na concentrations (Fig. 2)

Evidence for active uncoupled Na -transport

From the experiments described above it appears that the Na and K -transport is tightly coupled. This implies that Na -transport should cease as soon as the pump has depleted the liposomes of K Yet above a limiting Na -concentration in the incubation medium, net Na transport continues The Na K -pump appears to switch to uncoupled Na -transport as soon as the concentration at the K site has reached a low limiting level. The exact Na requirement to initiate uncoupled Na -transport is under investigation

The experiment shown in Fig. 3 was performed in an incubation medium containing 40 mM Na and 50 mM K ATP hydrolysis was monitored during net N K exchange (phase I) during uncoupled net Na transport (phase II) and at a steady state (phase III) In phase I 940 nmoles Na and 420 nmoles K were transported per mg protein for 400 nmoles ATP hydrolysed

From min 5 to min 25 (phase II) 540 nmoles Na were transported per mg protein and 550 nmoles ATP were hydrolysed after subtraction of the ATP consumption due to passive backleak. Thus the coupling ratio was about 1Na 1ATP When compared to phase I one more Na has been transported and at least one more ATP has been hydrolysed

When the Na -gradient reached steady state ATP hydrolysis became linear with respect to time (phase III) This was expected since at steady state the limiting factor for ATP hydrolysis is passive backleak of Na and K

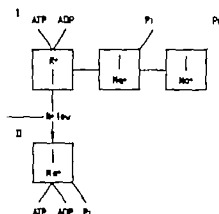


Fig 4 Simplified scheme of the properties and functions of the purified Na K -pump The upper case contains the functional transport unit I which catalyzes coupled Na K -transport *with a stoichiometry of 2.2Na* 1K 1ATP (phase I). The dotted line designs the non-obligatory linkage In the lower case the functional transport unit II which catalyzes active uncoupled' Na transport (phase II) at coupling ratio of 1Na 1ATP is presented When the liposomes are depleted of K after phase I the pump catalyzes transport of one more Na but without K -antiport

DISCUSSION

en Na K ATPase isolated from the renal out er medulla is reconstituted in phosphatidylcholine liposomes, a functional Na K pump is obtained In the present work the reconstituted Na K transport system was used to study the nature of the Na K coupling.

It was observed that the initial velocity of the Na K exchange process increases when the Na concentration in the incubation medium is increased at a constant K concentration. At 50 mM Na and 50 mM K for instance the pump turns over 8 times more rapidly than at 20 mM Na and 50 mM K yet the Na K coupling ratio is invariably around ...Na K At high turnover rate the number of ATP molecules hydrolysed appears to be equal to the number of K transported Accordingly the stoichiometry of the overall process is 2.2Na 1K 1ATP

The observation that the Na K coupling ratio is the same whether the pump turns over slowly at a low Na K ratio in the incubation medium or more rapidly at a high Na K ratio in the incubation medium may infer that the Na K coupling is rigid However experiment on Na K ATPase which has been selectively modified in its Na form by trypsin treatment have revealed that 60 % of the Na transport capacity can be suppressed without affecting the K transport capacity (Anner & Jorgensen 1979 Jorgensen & Anner 1979) Consequently countertransport of one Na for one K may be sufficient to sustain Na K exchange The dotted line in Fig. 4 symbolizes the link which is first exposed to trypsin in the presence of NaCl

In the present report it has been emphasized that a fraction of the active Na transport catalyzed by the Na K -pump does not require counter-transport of K This uncoupled' net Na transport starts when liposomes have been depleted at K The onset of uncoupled Na -transport requires a minimal concentration of Na in the incubation medium Uncoupled' Na transport discontinues when a maximal gradient of 3...Na 1K is reached

In conclusion the results reported herein infer that the purified Na K -pump contains two functional transport units one catalyzes coupled active Na K transport and the second catalyzes uncoupled' active Na transport. The simplified scheme shown in Fig. 4 illustrates how the transport units appear to be interacting' uncoupled' Na transport starts when the pump has lowered the K concentration at the K -site to a limiting level

A certain versatility of the Na K -pump has been anticipated from studies in intact cells (Glynn & Karlish 1975) The present work shows that versatility is inherent in the purified Na K pump and shows that the fundamental transport properties are preserved when the Na K transport system has been isolated and reconstituted in artificial membranes The protein part of the Na K -pump is composed of two 100 000 mol wt catalytic subunits and of two to four 40 000–60 000 mol wt glycoproteins It is reasonable to assume that the distinct transport patterns of the Na K pump are the expression of specific configurations or interactions of the subunits Therefore the reconstituted transport system will be a useful tool in the attempt to relate the physiological role of Na K ATPase with its molecular properties.

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Cholera toxin interactions with lipid bilayers

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The purpose of the experiments described in this paper was to assess the binding of cholera toxin to bilayers containing its receptor the monosialoganglioside, G_{M1} The assay was based on the fact that G_{M1} confers on the bilayer negative surface charge. The magnitude of this surface charge was estimated by measuring the electrical conductance (G) of the bilayers exposed to nonactin-K under conditions where G is directly proportional to the potassium concentration in the aqueous solutions immediately adjacent to the membrane surface When bilayers were formed from mixtures of G_{M1} and glycerolmonooleate (GMO) it was found that the molar ratio of the lipids in the bilayer was the same as that in the membrane forming solution. It was further found that cholera toxin or the binding subunit of the to in (choleragenoid) bind to GMO bilayers containing G_{M1} (but not to GMO bilayers containing phosphatidyl serine or disialoganglioside G_{D1a}) The value of the apparent dissociation constant for the binding of choleragen to its receptor was found to be 10^{-1} M comparable to values found in intact cells.

Work in many laboratories has shown that cholera toxin exerts its effect on cells through activation of the adenylate cyclase (as reviewed by Pierce et al 1971 and Finkelstein 1976) Choleragen is a protein of approximately 84 000 daltons containing two different subunits A and B The holotoxin ol gomer is composed of one A (27 000–29 000 D) and 4–6 B (10 000–12 000 D) subunits The A subunit consists of two peptide chains, A_1 (20 000–25 000 D) and A_2 (2 500–9 700 D) linked through a disulfide bond (Klapper et al 1976) The activation of the adenylate cyclase by cholera toxin in intact cells begins with the binding of the toxin to the cell surface receptor the monosialoganglioside G_{M1} The specific interaction of choleragen with the receptors is mediated through the B subunits of the protein (choleragenoid) (Cuatrecasas 1973 van Heyningen 1974) The subsequent events which lead to the time-dependent activation of the cyclase are less well understood It has been proposed that this lag period between exposure and cyclase activation which varies from 30 to 120 min depending on the type of cell under study may represent the time required for lateral motion of the toxin on the cell surface (Bennett et al 1975) penetration of the biologically active A peptide into or through the plasma membrane (Gill & King 1975) and the covalent modification of a cellular protein in an NAD-dependent ADP ribosylation reaction catalyzed by the A peptide (Gill 1975) The activation of the cyclase by choleragen would then proceed through this ADP-ribosylation of an as yet unidentified intracellular protein.

Our interest in cholera toxin was stirred by a report of Moss et al (1977) in which they showed that choleragen interacts with lipid vesicles promoting an increase in their glucose permeability We decided to study the possibility that the toxin–receptor interaction might lead to changes in the ionic permeability of membranes anteceding its effect on adenylate cyclase We have shown (Tosteson &

Abbreviations used GMO = glycerolmonooleate PS = phosphatidyl serine PC = phosphatidyl choline, G_{M1} = galactosyl-N-acetylgalactosaminyl-(N-acetylneuraminyl)-galactosyl-glucosyl-ceramide G_{D1a} = N-acetylneuraminyl-galactosyl-N-acetylgalactosaminyl-(N-acetylneuraminyl)-galactosyl-glucosyl-ceramide.

Tosteson 1978) that when cholera toxin was added to one of the aqueous solutions surrounding a planar bilayer made with glycerolmonooleate and G_{M1} it interacts with the bilayer promoting an increase in membrane conductance The magnitude of this increase was found to depend on the choleragen concentration in the aqueous solutions Using different concentrations of G_{M1} in the membrane forming solution, we have further shown that the toxin concentration required to produce half the maximal steady-state conductance increase 1.2×10⁻ M is very similar to the value found by Cuatrecasas (1973) for the interaction of choleragen with fat cell membranes Finally we have also shown that the increase in membrane conductance is associated with the formation of channels, with a unit conductance of 20–40 pmho

In this paper we describe experiments designed to measure the binding of holotoxin and choleragenoid to GMO bilayers containing 10% G_{M1} The results of these experiments show that both cholera holotoxin and choleragenoid bind to these bilayers and that the apparent dissociation constant for the process is 10⁻ M

ATERIALS AND METHODS

(a) M mbrane formation and electrical measurement

Bilayers were formed from GMO or from a mixture of GMO and G_{M1} in the molar ratios indicated, by apposition of two monolayers spread at the air-solution interface (Montal & Mueller 1972). The aqueous solutions present in the 1.5 ml chambers were buffered to pH 7 0 with 1 or 5 mM TrisCl. The contents of both chambers were stirred continuously with magnetic stirrers The steady-state membrane conductance was determined in the limit of zero applied potential by measuring the steady-state current flowing across the membrane in response to an applied potential difference using silver-silver chloride electrodes.

(b) Surface harge measurement

The surface charge density of GMO bilayers containing monosialogangliosides was determined using nonactin-K as a probe for surface potentials Bilayers were made from GMO + G_{M1} in the presence of 1 mM KCl and 6.6×10^{-4}–10^{-7} M nonactin buffered to pH 7 0 with 1 mM TrisCl After the membrane conductance (G) had reached steady value the ionic strength of the aqueous solutions was increased by additions of aliquot of a concentrated solution of LiCl a non-permeant cation, and G was determined after each addition The surface charge density σ_p, was then obtained by fitting the experimental point to the equation (McLaughlin et al 1970)

$$G = A\left[\frac{136\sigma}{\sqrt{\Sigma c_i}} + \sqrt{\left(\frac{136\sigma}{\sqrt{\Sigma c_i}}\right)^2 + 1}\right]^2 \quad (1)$$

where A is a constant (at constant K and nonactin concentrations) equal to the membrane conductance at infinite salt concentration, and c_i is the molar concentration of all ionic species.

(c) Binding of choleragen and choleragenoid

Binding of holotoxin or choleragenoid was determined through measurements of the change in the surface charge of the bilayers Bilayers containing monosialoganglioside were formed in the presence of 1 mM KCl 29 mM LiCl and 10⁻ M nonactin buffered to pH 7.0 with 5 mM TrisCl. After determination of the steady-state membrane conductance aliquots of cholera toxin (or choleragenoid) were added to both sides of the membrane and the conductance G_P, determined after each addition. The number of molecules of G_{M1} bound (σ_b) were then calculated according to the following equations Hladky & Haydon 1973)-

$$\ln G_P/G_s = -\frac{F}{RT}\Delta\phi_P \quad (2)$$

and

$$\sigma = \sigma - \frac{\sqrt{c}}{136}\sinh\frac{(\Delta\phi_s - \Delta\phi_P)F}{2RT} \quad (3)$$

where G_s is the membrane conductance prior to the addition of proteins, σ is the surface charge density obtained through equation 1 and $\Delta\phi_s$ is the interfacial potential due to this surface charge density at a total electrolyte concentration

(d) M terials

Glycerolmonooleate was purchased from Nu-Chek, PS and the mono- and disialogangliosides were obtained from Supelco Inc. and cholera toxin from Schwarz Mann. Choleragenoid was a gift from Dr R. A Finkelstein (Dept. of Microbiology University of Texas Southwestern Medical School, Dallas, Texas).

RESULTS AND DISCUSSION

(a) Membrane composition

Fig. 1 shows the results of experiments designed to estimate the surface density of G_{M1} molecules in the bilayers The fit of the experimental points to equation 1 (Methods) yields a value of 10 charges/Å for pure GMO membranes and 8.3×10^{-4} and 25×10^{-4} charges/Å for bilayers made from solutions containing G_{M1}. GMO molar ratios of 0.03 and 0 1 respectively Since there is one charge per molecule of G_{M1} and the area per molecule of GMO is 40 Å

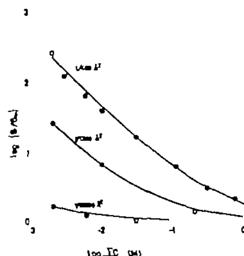


Fig. 1 Determination of bilayer composition. Bilayers were formed from pure GMO (curve labelled 1/10 000 Å²) or from GMO containing G_{M1} at 0.03 (curve labelled 1/1 200 Å²) or 0.1 (curve labelled 1/400 Å²) molar ratio in the presence of 1 m*M* KCl + 1 m*M* Tris-Cl pH 7.0, 6.6×10^{-8} *M* nonactin. The ionic strength was increased by addition of concentrated LiCl containing the same concentration of the other ions and nonactin as in the initial solution. Points are mean values obtained in 4 or more different membranes. The lines were drawn according to Equation 1 (Methods) with the value of σ as indicated for each line.

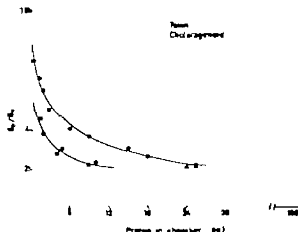


Fig. 2 Effect of choleragen (●) and choleragenoid (O) on the nonactin-K induced conductance G_{M1}+GMO 0.1 molar ratio) bilayers were formed in 1 m*M* KCl+29 m*M* LiCl+5 m*M* TrisCl (pH 7.0) in the presence of 10^{-} *M* nonactin. Stock protein solutions (choleragen: 5 mg/ml choleragenoid 0.5 mg/ml) were diluted prior to use with the initial medium and small portions were added to both aqueous phases bathing the bilayer. Volume of chambers: 1.5 ml. Points represent mean values obtained in at least 4 different membranes.

(Hladky & Haydon 1973) these charge densities correspond to 0.03 and 0.1 molecules of G_{M1} per molecule of GMO values which coincide with the composition of the lipid mixtures used to form the bilayers.

These results extend those of Hill & Lester (1972) who found that when gangliosides were rehydrated together with phosphatidylcholine the ratio of ganglioside to PC molecules in lamellae of a combined ganglioside-lecithin smectic mesophase was of the same composition as the initial mixture (up to molar ratios of 0.3)

(*b*) *Binding of cholera toxin and choleragenoid*

Fig. 2 shows the effect of cholera toxin and choleragenoid on the nonactin-induced K conductance of GMO bilayers containing 10% G_{M1}. The values on the ordinate are the ratios of the conductance in the presence to that in the absence of toxin (or choleragenoid). The results depicted in the figure show that addition of either of the proteins lowers the membrane conductance to 20% of the value in the absence of protein. This suggests that the interaction of these proteins with the ganglioside molecules in the bilayer lowers the surface charge density of the bilayer by about 80%. This estimate of the fraction of G_{M1} charges neutralized by cholera toxin (or choleragenoid) was confirmed by the results of the experiments described in Fig. 3. The residual surface charges remaining after addition of high concentrations of proteins was estimated by increasing the ionic strength of the bathing solutions. The value for the surface charge density obtained 8.3×10^{-4} charges/Å indicates that after exposure to toxin (or choleragenoid) the surface charge density of GMO + G_{M1} bilayers is 20–30% of the initial value.

The specificity of the toxin binding to its receptor was tested by changing the lipid mixture of the bilayers. It was found that addition of toxin (or choleragenoid) to GMO bilayers containing phosphatidyl serine (0.1 molar ratio) or disialoganglioside G_{D1a} (molar ratio 0.08) did not change the initial nonactin induced conductance of these bilayers. Changing the ionic strength of the solutions bathing these bilayers after addition of high

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Ion movement through gramicidin A channels

On the importance of the aqueous diffusion resistance and ion–water interactions

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ANDERSEN O S & PROCOPIO J Ion movement through gramicidin A channels. On the importance of the aqueous diffusion resistance and ion–water interactions. Acta Physiol Scand 1980 Suppl. 481 27 35 Department of Physiology and Biophysics, Cornell University Medical College, 1300 York Avenue, New York, NY 10021 USA.

The movement of an ion through a membrane channel proceeds in at least five separate steps. Diffusion through the aqueous phases up to the channel association with the channel itself translocation through the channel, dissociation from the channel, and diffusion through the aqueous phases out from the channel W demonstrate that contrary to current working assumptions, the aqueous diffusion step may be an important determinant of overall ion movement through the channel. W further describe the kinetics of Na movement through gramicidin A channels. Using these data we show that one will have to consider the movement of H_2O through the channel explicitly in any complete model for ion translocation through the channel interior

The molecular mechanisms by which integral membrane proteins permit specific solute permeation through the membrane are not known in detail But it is likely that the translocation of small inorganic ions—and probably most other polar solutes—proceeds through a pre formed path lined with polar groups which spans part or all of the hydrocarbon core of the membrane If this structure forms a continuous pathway from one aqueous phase to the other one may call it a channel

The permeability properties of a channel will among other factors depend upon its dimensions. In one extreme case the channel can be looked upon as a large water filled structure through which ion movement proceeds essentially as a free electro-diffusion process. The selectivity among ions of the same valency will largely be determined by the aqueous mobility of the ions. Selectivity among ions of different valency will in addition, be influenced by electrostatic factors. In the other extreme case the channel is a quite narrow structure an array of coordination sites which allow the movement of ions and water to occur as a series of rapid jumps from one potential energy minimum to an adjacent—with pauses for various times at each site (potential energy minimum) The movement of ions and water proceeds in an orderly fashion as neither ions, H_2O molecules nor ions and H_2O molecules can overtake each other the channel exhibits *single-file* behavior (Hodgkin & Keynes 1955 Heckmann 1965*a* Hille & Schwarz 1978 Finkelstein & Rosenberg 1979) In biological membranes an example of the first channel type is the acetylcholine activated channel in the neuromuscular junction (Dwyer et al. 1979) An example of the second channel type is the delayed rectifier (K) channel in nerve and muscle (Hodgkin & Keynes 1955 Armstrong 1975 Hille & Schwarz 1978 Begenisich 1979)

The fact that one can divide channels in biological membranes into several different categories, does not imply that one understands the molecular mechanisms involved in ion translocation or ion

It is not known whether ions move by a single-file mechanism relative to water in the K channel. The dimensions of the K channel (Hille 1975*b*) make this likely possibility The electro-osmotic potentials observed by Vargas (1968) may indeed reflect ion/water single-filing.

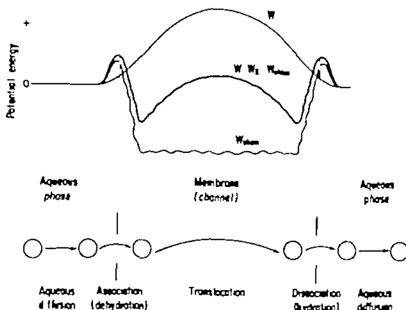


Fig 1 Schematic representation of a model for ion translocation through the Gram A channel Top. The potential energy profile. W_{chem} denotes the qualitative variation in the local interactions between the ion and the wall. W denotes the variation in the electrostatic image energy as an ion moves through the channel (Levitt 1978). The potential energy profile W is the sum of W + W_{chem}. Bottom. A schematic illustration of the five separate steps involved in the movement of an ion from one *aqueous phase to the other*

specificity in detail. Progress towards this goal will ... y be slow in view of the unknown and probably quite complicated, structure of these channels. Studies on channel-mediated permeability phenomena in artificial lipid bilayers are therefore likely to contribute significantly to a deeper *understanding of ion movement through channels in* biological membranes. Examples of wide water filled channels are the polyene-channels (Finkelstein & Holz 1973 Andreoli 1974). An example of a narrow channel exhibiting single file properties is the gramicidin A channel (Levitt et al. 1978, Rosenberg & Finkelstein 1978*a* Schagina et al 1978 Procopio & Andersen 1979)

GRAMICIDIN A

Gramicidin A (gram A) forms ion-selective channels which span lipid bilayer membranes (Hladky & Haydon 1972). The chemical structure of the channel is known (Sarges & Witkopf 1965) and considerable evidence is available concerning the conformation of the channel (Urry et al 1975 Bamberg et al. 1977 *Veatch & Stryer 1977* Koeppe et al. 1978) Gram A channels are cation selective (Myers & Haydon 1977 Urban et al 1978 but see Eisenman et al 1977) and exhibits a respectable selectivity among monovalent cations (Myers & Haydon 1972 Eisenman et al 1978 Urban et al 1978) Gram A channels further exhibit *mole-fraction-dependent conductance* changes (Andersen 1975 *Neher 1975 Eisenman et al 1977 Andersen 1978* McBride & Szabo 1978 Neher et al 1978) Gram A channels thus exhibit permeability properties which are phenomenologically similar to what can be observed in channels in excitable membranes (Hagiwara & Takahashi 1974 Cahalan & Begenisich 1976) There are a number of important differences between the Gram A channel and channels in biological membranes most notably the absence of fixed charges in the channel itself Studies of the permeability properties of the very simple gram A channel should nevertheless be able to contribute significantly to the understanding of channel-mediated permeability phenomena in biological membranes

PERMEABILITY

Ion fluxes through channels have traditionally been described in terms of an electrodiffusion formalism.

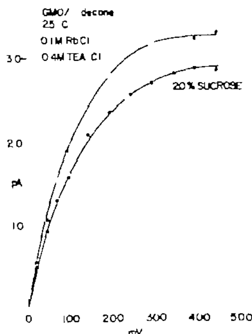


Fig 2 Current-voltage characteristics of Gram A single channels in glycerolmonooleate/n-decane membranes The aqueous phases contain symmetrical 0 1 *M* RbCl+0 4 *M* tetraethylammonium chloride with (O) or without (●) 20% (w/v) sucrose. The points are the mean values of between 115 and 1037 current transitions at different potentials. The standard deviations are always less than 14% of the mean values in all cases. The conductivity of the aqueous phases decreases by 28% upon the addition of sucrose T=25°C.

either the integrated version of the Nernst Planck flux equations as obtained by Goldman (1944) and Hodgkin & Katz (1949) or the equivalent circuit formalism of Hodgkin & Huxley (1952) Finkelstein & Mauro (1963) should be consulted for an incisive analysis of both formalisms Both the above approaches assume free movement of ions and water relative to one another which may be adequate to describe ion movement through wide channels. But such formalisms are unable to describe transport mechanisms that exhibit more complicated behavior such as saturation of the flux competition among several permeant or impermeant ions concentration-dependent ion selectivity and coupling among the fluxes of several species (Hille 1978)

Alternative models for ion translocation through narrow channels have therefore been constructed on the basis of a finite one-dimensional random walk model with transition probabilities which may depend upon position, applied potential and the number (and type) of ions which may occupy the channel at any time (Hodgkin & Keynes 1955 Heckmann 1965*a* *b* Läuger 1973 Markin & Chizmadjev 1974 Hille 1975*a* *b* Aityan et al 1977 Sandblom et al. 1977 Hille & Schwarz 1978 Levitt 1978*b* Hägglund et al 1979 Urban & Hladky 1979) The essential features of such models are illustrated in Fig. 1 The potential energy profile possesses a minimum near each of the channel openings. the channel possesses two sites[a] in the single-file region—but it may have additional binding sites exterior to the channel proper The two potential energy minima are separated from each other by potential energy barriers. The movement of an ion over each of the barriers can be represented by a series of discrete jumps which can be described by the absolute rate theory (Parlin & Eyring 1954) Alternatively one may regard each of the steps to occur as a single jump—in which case the strict applicability of the absolute rate theory becomes problematical In both situations one can make the rate of crossing the various barriers to depend upon the number of ions occupying the channel. At present neither formalism considers explicitly the movement of any H_2O molecules which occupy the channel—and may constrain the ion movement.

The present models have been developed using a number of simplifying assumptions some of which may be incorrect. Firstly it is generally assumed that any limitations imposed by the aqueous diffusion step can be disregarded Secondly it is usually assumed that the ions move by a *vacancy* mechanism in which the ions only can move into a site that has been vacated by its previous occupant. This may not be a reasonable assumption in a channel that only contains a few ions (and H_2O molecules) It is possible that the ions also can make use of the *knock-on* mechanism in which the ions move by "colliding with each other thus causing the displacement. This mechanism could become particularly pronounced when a potential difference is applied across the membrane The resulting flux equations will differ appreciably at high occupancies of the channel (Hodgkin & Keynes 1955 Heckmann & Vollmerhaus 1970) Thirdly in channels where ions and water are constrained to move in single-file do the ions and water relative

[a] This usage of the word site differs ... that introduced earlier

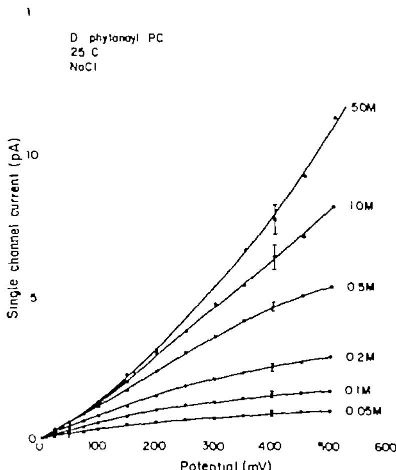


Fig 3 Current voltage characteristics of gram A single channels in diphytanoylphosphatidylcholine/n-decane membranes The aqueous phases contained symmetrical NaCl at the concentrations indicated The points are mean values of 68 to 2261 (usually 100–800) current transitions of each potential. The vertical bars at 400 mV indicate the standard deviations at these potentials The standard deviation is always less than 20% (usually less than 10%) of the mean value. T=25°C

to each other move by a *vacancy* or a *knock-on* mechanism—and do they stay in the same mode at all applied potentials and ion occupancies? If the ions relative to H_2O move in the *vacancy* mode then it should be reflected in a very weak voltage-dependence of the rate constant for the individual steps It further becomes necessary to consider the role of any H_2O molecule which separate two ions when deciding whether to use the *vacancy* or the *knockon* mode to describe the ion movement in such a multiply occupied channel

We presently demonstrate that contrary to current working assumptions the aqueous diffusion step may be an important determinant of the observed kinetics of ion movement through the membrane *and* that one probably will have to consider the H_2O movement through the channel explicitly in any successful model for ion translocation through the gram A channel

AQUEOUS ACCESS LIMITATION

The shape of the current voltage characteristics of gram A single channels depends upon the aqueous concentration of the permeant ion (Hladky & Haydon 1972 Hägglund et al 1979) see also Fig. 3 At low concentrations the single channel current reaches a limiting value I_{lim} with increasing potential as illustrated in Fig. This indicates that the ion movement is limited by a voltage independent step which can be unmasked at permeant ion concentrations sufficiently low that the channel is far

from saturation Such limiting currents are most easily demonstrated using very permeant ions such as Rb but they can also be inferred for less permeant ions such as Na see Fig. 3 The magnitude of I_{lim} is about 3.2 pA in 0 1 *M* RbCl+0 4 *M* TEA Cl This value is consistent with a diffusion-controlled movement of RB up to the channel—which is represented as a hemi-spherical sink in a plane interface with a capture radius[1] of approximately 0 05 nm (Läuger 1976) The theoretically calculated value for I_{lim} is about 5 pA in reasonable agreement with the measured value of about 3. pA—considering the approximations made in the calculations

If the magnitude of I_{lim} is determined by diffusion through the aqueous phases up to the channel one would predict that an increase in the aqueous viscosity would cause a decrease in the magnitude of I_{lim} This is indeed the case as illustrated in Fig. 2. When the aqueous phases contain 20% sucrose which almost doubles the viscosity I_{lim} decreases to about 2.8 pA. The relative decrease in I_{lim} is 45% of the decrease in aqueous solution conductivity This discrepancy is expected as the major diffusion resistance occurs with 0 1–0.2 nm of the channel (Andersen 1980) a distance much smaller than the dimensions of the sucrose molecule.

From the magnitude of I_{lim} one can calculate the magnitude of the convergence permeability up to the channel. 3 4×10^{-14} liters/(channel × sec) for Rb The convergence conductance of each of the aqueous phases is therefore 125 pS (Läuger 1976) This value should be compared with the small-signal single channel conductance 22 pS Only 2/3 of the measured single channel resistance originates in the channel itself Increasing the viscosity of the aqueous phases will therefore be expected to decrease the single channel conductance at all potentials as observed in Fig. 2. At low potentials the observed decrease is however larger than predicted.

Experiments with the alkal metal ions and NH_4^+ show a uniform pattern–the magnitude of the single channel currents at high potentials (450–550 mV) is consistent with a diffusion-limited ion entry into the channel (Andersen 1978 Andersen & Procopio 1979*b*) Two ions stand out from this pattern Ag and Tl The currents at 500 mV do not show definite signs of reaching their limiting value, and are more than two times larger than predicted from the alkali metal ion experiments (Andersen 1978 Andersen & Procopio 1979*b*) This finding implies that the effective capture radius of the gram A channel has increased—by 100% or more Such an increase could be caused either by an increase in the channel diameter per se—as a result of a structural rearrangement, or by the existence of additional binding sites exterior to the channel proper The ions could move from these binding sites into the channel by a mechanism resembling lateral diffusion. The general features of such a mechanism have been analyzed by Adam & Delbrück (1967) who found that it can increase the effective capture radius several fold

One can distinguish between the two possible mechanisms by which the effective capture radius is increased by electrical measurements (Andersen 1980) or by using small non-electrolytes as probes of the channel dimensions The interpretation of *changes* in permeability coefficients for a permeable solute is however very difficult in a single-file channel, as the permeability will be affected by changes in channel dimensions as well as by blocking of the channel by ion bindings as described by Dani & Levitt (1979) But the gram A channel is normally impermeable to urea (Finkelstein 1974) while it is permeable to the only slightly smaller formamide (Procopio & Andersen, unpublished observations) *If* the increase in effective capture radius reflects an increase in the luminal diameter of the channel this should be registered as a measurable urea flux through the channel This was not observed see Table 1 We find that the unmodified membrane permeability for urea is unaffected by the composition of the aqueous phases, and that there is no detectable urea flux through the gram A modified membranes irrespective of the cation present. We conclude that Ag and Tl do not produce *major* changes in channel structure. A similar result has been obtained from studies of the single channel currents of various mole fractions of Ag or Tl^+ and Cs (Andersen 1980) These investiga-

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The capture radius, r_c, is the difference between the channel radius, R, and the ion radius, r (Ferry 1936, Läuger 1976). The relevant magnitude of each of these will be determined by the detailed mechanism through which the ion reaches the channel mouth—especially how the hydration water is handled. A likely maximum estimate for r_c can be obtained as the difference between the luminal radius of the channel, about 0.2 nm (Urry et al 1975) and the crystal radius of Rb 0 15 nm (Hille 1975*b*)

Similar results have been obtained by Tredgold & Jones (1979) who changed the viscosity of the aqueous phases by substituting the more viscous D_2O for H_2O

Table 1 *Urea permeability of lipid bilayers*

Mean ±S.D. The number in parenthesis indicates the number of xperiments. Di-phytanoylphosphatidylcholine/n-decane membranes. 25°C *All solutions contained* 10 mM urea. The conductance of the gramicidin A modified membranes was larger than 1S/cm²

Salt	Without gramicidin A (cm/sec)	With gramicidin A (cm/sec)
NaCl 1.0 *M*	6.0±0 7×10⁻⁷ (4)	5±2×10⁻ (4)
$AgNO_3$ 1 0 *M*	6±2×10⁻⁷ (3)	6×10⁻ (1)
$TlNO_3$ 0.3 *M*	8±4×10⁻ (2)	6±1×10⁻ (2)

tions have additionally provided more direct evidence for the binding of Ag and Tl to some outer bindings sites

There is, however some evidence that the molecular behavior of the gram A channel may be altered by Ag and Tl relative to what is observed with the alkali metal ions and NH_4 The average channel lifetime is about 5 times longer in the presence of Ag and Tl than in the control situation (Andersen & Procopio 1979*b*) It is presently not known whether the intrinsic permeability properties of the channel are specifically affected by Ag or Tl —apart from the effects brought about by multiple ion occupancy and occupancy-dependent rate constants for moving across the various barriers.

ION MOVEMENT THROUGH THE CHANNEL

The details of the kinetics of ion movement through conducting channels can be obtained by analyzing the single channel current voltage characteristics over a range of aqueous salt concentrations and as large potential variation as technically possible Fig. 3 illustrates such data, obtained with Na as the current carrier It should be noted that (*a*) at low salt concentrations the current tends to reach a limiting value with increasing potential—reflecting the importance of the aqueous diffusion step and (*b*) at high salt concentrations the currents increase "exponentially with increasing potential—reflecting the permeability properties of the channel itself

A kinetic analysis of these data is greatly facilitated by the observation that the movement of ²²Na through the channel can be described by the Ussing (1949) flux-ratio equation (Procopio & Andersen 1979) This may indicate that there can be no more than one Na in the channel at attainable salt concentrations although it is possible that the channel could be occupied by two Na If the major barrier for ion movement through the channel is the translocation step across the central barrier in Fig 1 (Heckman 1965*b* Hille & Schwarz 1978 Urban & Hladky 1979)

Details of the kinetic analysis and of the control experiments will be given elsewhere The results indicate that it is the former possibility (only one ion) which is correct—although small systematic discrepancies between the predictions of simple model and the observed data occur at very high salt concentrations (Andersen & Procopio 1979*a*) We believe that these discrepancies reflect the variation in water activity which occurs at very high salt concentrations Table 2 summarizes our results for gram A in bacterial phosphatidyl-ethanolamine/n-decane membranes. We also list the water permeability obtained from Rosenberg & Finkelstein (1978*b*) It should be noted that the rate constant for translocation through the channel interior is larger than the rate constant for exit out of the channel Ion movement through the channel is limited by the exit step contrary to intuition which would locate the major barrier in the channel interior A qualitatively similar result has been found for gram A channels in glycerolmonooleate/n-decane and diphytanoylphosphatidylcholine/n-decane membranes The position of the potential energy minima appears to be very similar for all three lipids, about 65% of the applied potentials falls across the central barrier

It is known that Na and H_2O cannot pass each other in the gram A channel (Levitt et al 1978 Rosenberg & Finkelstein 1978*a*) But it is not known whether the Na movement is limited by the movement of Na *per se* or whether it is limited by H_2O movement It is therefore of interest to compare the rate constant for Na translocation through the channel interior to the rate of H_2O translocation which can be estimated to be about

The chemical identity of these outer sites is presently unknown. They are part of the gram A channel itself as their existence has been inferred in measurements made on channels incorporated into very different membranes, e.g. glycerolmonooleate/n-decane and diphytanoylphosphatidylcholine/n-decane membranes.

Table 2. *Kinetics of Na movement through gramicidin A channels*

Bacterial phosphatidylethanolamine/n-decane membranes.
23 ± 1°C.

Convergence permeability	$\sim 10^{-14}$ liters/sec
Maximal conductance	14.6 pS
Activity for half-maximal conductance	0 23 m
Dissociation constant for a single site	0.30 m
Rate constant for the dissociation step	1.2×10^7 ions/(channel × sec)
Rate constant for the association step	7×10^{-17} liter/(channel × sec)
Rate constant for the translocation step	$\times 10^7$ ions/(channel × sec)
Diffusional water permeability*	$\times 10^{-14}$ cm^3/sec

From Rosenberg & Finkelstein (1978 b).

6.7×10^7 H_2O molecules/(channel × sec) using the permeability coefficient listed in Table 2 The gram A channel contains approximatelh 5–6 H_2O (Rosenberg & Finkelstein 1978 *a b*) If one assumes that the electrical distance separating the two potential energy minima approximates the real distance then we find that 3–4 H_2O molecules separate the two potential energy minima We thus predict the rate constant for Na translocation through the channel interior to be about 2×10^7 ions/(channel × sec)—which is the independently determined value It is therefore very likely that the Na movement is water-limited, but we do not know whether ions and H_2O move by the *vacancy* or *knock-on* mode at low potentials.

This correlation indicates, nevertheless that the magnitude of the central potential energy barrier W may be considerably smaller than indicated in Fig. 1

At high potentials it appears that ions can push H_2O ahead of itself out of the channel. At 500 mV and 5 0 *M* NaCl the single channel current, in bacterial phosphatidylethanolamine/n-decane membranes, is about 11 pA and shows no sign of reaching a limiting value This current corresponds to a movement of 7×10^7 ions/(channel × sec) which corresponds to about 4×10^8 H_2O molecules/(channel × sec), or 6 times the unidirectional H_2O flux at zero potential A simple argument based upon the concept of first passage times (Feller 1967) shows that the largest water flow which can be associated with ion movement through a single-file channel operating in the *vacancy* mode is about 3 times the unidirectional water flux at equilibrium measured by tracer 7×10^7 H_2O/(channel × sec) (Andersen manuscript in preparation) The observed Na movement is thus about two times larger than the upper limit for a single-file channel in the *vacancy* mode. The discrepancy is significant, but not glaringly so It is therefore important to note that currents greater than 20 pA have been observed with NH_4^+ as the current carrier At high potentials ions can indeed knock H_2O molecules out of the channel In retrospect, this conclusion is not surprising as an ion gains a potential energy of about 20 kT if the potential is 500 mV k is Boltzmann's constant, T is temperature in K The present data still leave open the question of whether ions and H_2O at low potentials move in the *vacancy* or *knock-on* mode, as well as the question of ion–ion interactions.

We wish to thank Cheryl K. Martin and Frank I Navetta for excellent technical assistance and Rochelle Lester for secretarial assistance.

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Modulation of alamethicin induced conductance by membrane composition

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The effect of cholesterol on the opening and closing of the alamethicin-channel was studied varying the cholesterol content of glycerolmonooleate membranes. Increasing the cholesterol mole fraction in the membrane shifted the log of the steady-state conductance-voltage curve to the right along the voltage axis. The shift was found to be 80–100 mV when the cholesterol mole fraction was increased from 0 to 0.5 The kinetic for the approach to steady-state conductance during voltage clamp was also studied in membranes under these conditions. At all the different cholesterol contents the on kinetic is well described by a single exponential The off kinetic on the other hand, is described by double exponential time course At the same voltage the time constant describing the on current relaxation is the same as the one found for the slow relaxation present in the off relaxation. Increasing the membrane cholesterol content increases the magnitude of the time constant describing the fast and slow process by several orders of magnitude. Cholesterol also increases the voltage dependence of the slow time constant. The effect of cholesterol on the steady-state conductance can be explained, but not in unique way by an increase in the membrane dipole potential. Phenomenological comparison of the fast and slow kinetic processes seen in multi-channel membranes with single channel characteristics indicates that (i) cholesterol increases the mean lif of the single channel and (ii) cholesterol increases the mean life of the different conductance levels.

As pointed out by Hodgkin & Huxley (1952) voltage-dependent conductances (permeabilities) should involve the movement of charged or dipolar molecules in the membrane field. "Gating currents due to this charge movement have been actually observed in several biological preparations, and are thought to be due to the molecular events preceding the opening of the sodium channel (Armstrong & Bezanilla 1974 Keynes & Rojas 1974 Nonner et al 1978 Bullock & Schauf 1978) Several fanciful models of the sodium channel have been proposed but whatever the actual structure of the channel is the movement of the voltage sensor (gating particles) should be affected by changes in membrane composition. For example alterations in lipid composition may affect the extent of membrane depolarization necessary to open the channels and/or the rates at which the channels open and close At present, it is very difficult to study the effect of lipid composition on voltage-dependent conductance in biological membranes because modification of the lipid composition of those membranes is not an easy task and also because the molecular architecture of a natural" voltage-dependent channel is not known.

In order to provide an insight into the mechanisms by which membrane composition modulates the opening and closing of voltage-dependent channels we have studied alamethicin-treated planar lipid bilayer membranes

The ideal system in which to study the effects of membrane composition on channel gating is one in which the membrane composition is well defined and in which the mode of action and molecular structure of the ionophore are well understood. Alamethicin-treated glycerolmonooleate cholesterol membranes approach this ideal. Alamethicin is a well characterized polypeptide both from the point of view of its molecular structure and from its mode of action in bilayers. On the other hand,

glycerolmonooleate membranes have been used extensively in bilayer studies and the influence of cholesterol on ion transport in this type of membranes has been well characterized (e g. Gordon & Haydon 1972 Szabo 1974)

Alamethicin is a linear polypeptide antibiotic isolated from *Trichoderma viride* consisting of 19 aminoacids of which 14 are hydrophobic (e g. Martin & Williams 1976 Pandey et al 1977) The main fraction of natural alamethicin is the so-called R_f30 fraction containing one titratable group with a pK of 5.5 which may correspond to the free glutamine or glutamic carboxyl end (Payne et al 1970 Pandey et al 1977)

Mueller & Rudin (1968) were the first to show that alamethicin is able to endow lipid bilayer membranes with a strongly voltage-dependent conductance The alamethicin-induced conductance (G) increases e-fold every 4-6 mV similar to the maximum sodium and potassium conductance in nerve and is also strongly dependent on both salt and alamethicin concentrations Restricting ourselves to positive potentials the conductance can be represented by

$$G = g_0[Ala]^{\delta}[salt]^{\epsilon}\exp(\alpha eV/kT) \quad (1)$$

where g_0 is a constant V is the applied potential and e k and T have their usual meanings. In phosphatidylethanolamine-decane and in glycerolmonooleate-hexadecane membranes $\delta=9$ $\epsilon=4$ and $\alpha=6$ (Eisenberg et al 1973 Gordon & Haydon 1975)

Alamethicin increases the conductance of planar lipid bilayer membranes by the formation of pores which do not have a fixed conductance but rather fluctuate between several conductance states (Gordon & Haydon 1972 Eisenberg et al 1973 Boheim 1974) The opening and closing of these pores are voltage-dependent. The different conductance states are not integral multiples of a unit conductance and have mean life times in the range of milliseconds whereas the mean life time of the pore may span several seconds (Gordon & Haydon 1972 1975 1976 Eisenberg et al. 1973 Boheim 1974 Hall 1975 Boheim & Kolb 1978 Kolb & Boheim 1978)

We present here the results of the effect that increasing the mole fraction of cholesterol in glycerolmonooleate membranes has on the alamethicin-induced conductance Inasmuch as the solvent in solvent-containing membranes may obscure any effect of lipid composition on this voltage dependent conductance we have minimized the amount of solvent in the membrane by forming the bilayers by apposition of two monolayers (Montal & Mueller 1972) The results are discussed in terms of the different models proposed for the alamethicin channel

MATERIALS AND METHODS

The procedure for forming the membrane by apposition of two lipid monolayers, as well as the circuit for measuring the electrical properties of the membranes, has been reported previously (Donovan & Latorre 1979) The lipids used [glycerolmonooleate (GMO Nu-Check Prep. Elysian, Minn) Cholesterol (Chol Anspec Co Ann Arbor Mich.) and phosphatidylethanolamine (PE, Supelco, Bellefonte Penn.)] were stored at −50°C in $CHCl_3$ solutions A portion of this stock solution was evaporated under a stream of nitrogen and redissolved in pentane at a concentration of 1–5 mg/ml. These lipid solutions were prepared fresh every day GMO-Chol mixtures were prepared by mixing the appropriate volumes of the chloroform-lipid solution with the subsequent evaporation of the $CHCl_3$ and redissolution in pentane to a final total lipid concentration of 1–5 mg/ml.

Steady-state experiment

Our experiments were performed with the pure R_f30 fraction of alamethicin which was kindly supplied to us by Dr J E. Hall After membrane formation the antibiotic was added to only one aqueous compartment (*cis* side). The *cis* side is defined as the positive side of the membrane, and positive current is the flow of cations from the *cis* to the *trans* side As discussed in detail by Boheim & Kolb (1978), the behavior of the alamethicin system depends, to a certain extent on the pretreatment with respect to membrane voltage and temperature We also found that the steady-state conductance drifted for about one hour before stabilizing therefore, in all experiments, current measurements were not begun until at least one hour after the addition of alamethicin. To obtain the dependence of conductance on alamethicin concentration membranes were formed in 1 *M* NaCl and a small amount of alamethicin was added. After one hour the conductance was measured at different potentials, and another small addition of alamethicin was made This procedure was repeated after each new addition of alamethicin. To obtain the dependence of the conductance on salt concentration the membrane was formed in 20 m*M* NaCl alamethicin was added and, after one hour the conductance measured Small portions of a 6 *M* NaCl solution (unbuffered pH ~5.5) were added symmetrically to the membrane and the conductance measured each time

Kinetics

To measure the kinetic response of multi-channel membranes to a voltage pulse membranes were formed in 1 *M*

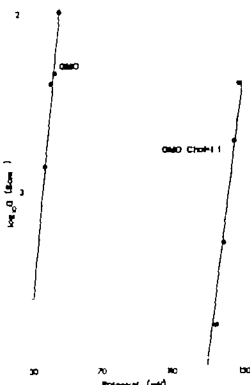


Fig. 1 Steady-state conductance-voltage curves for the alamethicin-induced conductance in GMO and GMO-Chol (molar ratio 1 1) membranes the conductance increases fold every 6 mV in both cases [Ala]=600 ng/ml, [NaCl]=1 0 *M* T°=22±1°C.

NaCl and R30 alamethicin was added. After one hour voltage pulses were applied starting from low potentials and proceeding to high potentials. The order was then reversed No difference was seen between two such pulses of the same potential. Generally from 4 to 3 current relaxations were summed to improve the signal to noise ratio

RESULTS AND DISCUSSION

General

The voltage-dependent ion conductance induced by alamethicin has been the subject of several studies (e g. Boheim 1978 Hall 1978) but a systematic study of the effect of lipid composition has not been done. Gordon & Haydon (1975) have reported an effect of lipid composition in solvent-containing bilayers on the rate constant for the transitions between the different conductance level of the alamethicin channel They found that the rate constants are very sensitive to the lipid composition, but the only conclusion that they were able to draw from their results was that membrane "fluidity" is involved in some way

At least two problems must be considered when comparing effects of lipid composition on carrier and channel mediated transport in solvent-containing membranes First, Pagano et al (1977) have shown that the bilayer composition of GMO-cholesterol-decane membranes is different than the composition of the membrane-forming solution such that the molar ratio of cholesterol to GMO in the membrane is about one half of that in the membrane forming solution Second, the ratio of lipid/hydrocarbon solvent in the bilayer appears to be a function of the lipid concentration and the membrane forming solution (Waldbillig & Szabo 1978) We think, therefore that a comparison between the mode of action of an ionophore in solvent-containing bilayers of different lipid composition should be viewed with caution as they may contain widely different amounts of solvent.

Because they contain very small amounts of solvent, membrane made by apposition of two monolayers are more suitable for this kind of study (e.g Reyes & Latorre 1979) Further indirect evidence will be given below that indicates that the bilayers formed with this method actually have approximately the same composition as the monolayers from which they are formed

Changes i steady-st te alamethicin-induced conduct nce due to th presence of cholesterol in the bilayer

Fig. 1 shows the effect of increasing cholesterol content of GMO membranes on the alamethicin-induced conductance. As can be seen in the figure cholesterol shifts the log G vs. V curve to the right along the voltage axis by more than 100 mV Thus, at constant salt and alamethicin concentration in the aqueous phases the voltage needed to open a given number of channels is increased with increasing cholesterol It is apparent from Fig 1 that cholesterol does not change the voltage dependence of the conductance induced by alamethicin The effect of increasing the mole fraction of cholesterol in GMO-Chol membranes is summarized in Table 1 The proportionality constant g in equation 1 sets the magnitude of G at given values of [Ala] [salt] and V For convenience we set these at [Ala] = 1 000 ng/ml [salt] = 1M and V = 100 mV The results shown in Table 1 indicate that changing the chole

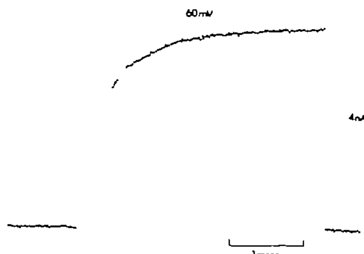


Fig 2 The alamethicin-induced current in a GMO membrane in response to a voltage step from zero to +60 mV The slight delay is not visible in this picture [Ala]=250 ng/ml, [NaCl]=1 *M* Membrane area 4×10⁻⁴ cm² T=22±1°C

sterol content of GMO membranes has little or no effect on the voltage dependence of the donduct ance and it only affects the absolute magnitude of the conductance

The large change in g_+ (~10 -fold) may have al conceivable causes. First variations in membrane lipid composition may change the adsorption coefficient of alamethicin in the membrane i e due to the high power dependence of the conductance on alamethicin concentration, a 10-fold change in adsorption coefficient would be enough to explain the change in g_+ Second changes in membrane viscosity could contribute to the change in g_+ It is known (Finkelstein & Cass 1967) that cholesterol lowers the water permeability of lecithin bilayers by about 10-fold Under our experimental conditions Donovan (1979) found that the acetamide permeability decreases by a factor of 6 when the mole fraction of cholesterol in the membrane changes from 0 to 0.5 (GMO membranes) Third the channel formation may be impaired for electrostatic reasons Szabo (1974) has shown that increasing the mole fraction of cholesterol in GMO bilayers increases the lipophilic anion-induced conductance but decreases the lipophilic cation-induced conductance Inasmuch as GMO and cholesterol are neutral molecules at the pH used in Szabo s experiments the most likely explanation for his results is that cholesterol increases the dipole potential of the membrane by as much as 100 mV We have performed similar experiments on membranes made by apposition of two monolayers Our results indicate that cholesterol increases the dipole potential in these membranes as well However in contrast to Szabo s results we find that the conductance changes for anions and cations are not symmetric the increase in anion conductance is less than the decrease in cation conductance at any given fraction of cholesterol in the membrane This indicates that the dipole potential is not the only factor determining the conductance changes. Other factors, such as mem-

Table 1 *Parameters of steady state alamethicin induced conductance in GMO and GMO-Chol membranes*

The parameters describe the steady-state alamethicin-induced conductance according to the equation

$G = g_+ C^{\delta}_{ala} C_{salt} \exp(\alpha e V/kT)$

The membrane area was 1 10⁻ cm²

Lipid	g_+(nS)	δ		α
GMO	3 9×10⁶	11 6±1	5 9_0 5	4 4±0.5
80%GMO–20%Chol	7 9 10⁴	1_3±1		4 1±0.5
70%GMO–30%Chol	2.8 10⁶			4.4_0.6
50%GMO–50%Chol	_8 10⁻⁴	10.6±1	5.2±0 7	4 1±1 0

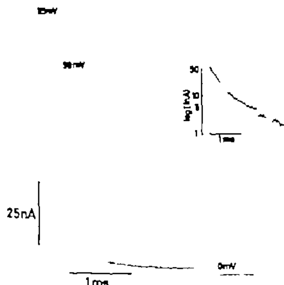


Fig. 3 The "off" response of the alamethicin-induced current in GMO membrane. A voltage pulse of 115 mV was applied, and the current allowed to come to steady state. The figure shows the end of the 115 mV pulse. The potential was then stepped to 90 mV. Finally the potential was stepped to 0 mV. The inset shows a semi-log plot of current versus time. Two exponentials are apparent, with $=0.3$ mS and $=0.085$ mS. The amplitude of the fast relaxation is 36 nA and that of the slow relaxation is 24 nA. Membrane area 2×10^{-4} cm²

brane viscosity as suggested by the acetamide permeability experiments must also be involved.

Steady-state measurements alone cannot distinguish between the different possibilities given above and therefore we have performed kinetic measurements in order to provide a further insight into the mechanism(s) by which cholesterol alters the alamethicin-induced conductance

Effect of cholesterol on the kinetic characteristics of the alamethicin channel

The record in Fig. 2 taken in an alamethicin-treated GMO membrane shows that if the voltage is suddenly changed from 0 to a sufficient positive value, the alamethicin-induced current increases monotonically to a steady state value. The time-course of the current is well described by a single exponential with a time constant ( ) of 0.4 ms. Exponential time courses for the alamethicin induced current have been reported previously for alamethicin-treated PE and phosphatidylcholine membranes can also be obtained from the alamethicin "off" kinetics. Fig. 3 shows the alamethicin "off" kinetics in a GMO membrane. The relaxation consists of an "instantaneous" ohmic drop in current and the time course of the current drop that follows the ohmic drop is well described by two exponentials, a fast relaxation with time constant τ and a slow relaxation with time constant τ_f. In GMO membranes τ is about 6–10-fold greater than τ

Increasing the cholesterol content of the membranes has a great effect on both τ and τ_f. In Fig. 4 is plotted as a function of potential for membranes made of GMO, 80%GMO–20%Chol and 50%GMO–50%Chol. τ was obtained from both on and off relaxations. Fig. 4 shows that increasing cholesterol has two effects on the time constant for the slow relaxation. First, it increases the magnitude of τ and second, it increases the voltage-dependence of . Cholesterol also slows down the fast time constant (Fig. 5) but it is difficult with the present data to extract a definitive conclusion about whether cholesterol is also modifying the voltage dependence of the fast process. It is noteworthy that both τ and τ are independent of alamethicin concentration (Fig. 4 and 5)

Recently both Boheim & Kolb (1978) have presented a detailed analysis of the alamethicin kinetics in dioleoylphosphatidylcholine-decane membranes. They made a comparison between the ob-

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The calculated dipole potential change in bilayers made from monolayers is 80 mV when the cholesterol mole fraction in the lipid solution used to form the monolayers is increased from 0 to 0.5. This calculation has been done assuming that ions of similar structure like $TPhA_s$ and $TPhB^-$ will be affected to the same extent by changes in membrane fluidity. For the same change in cholesterol mole fraction in the membrane forming solution, Szabo (1974) obtained dipole potential change of 30 mV in GMO-decane membranes. This result is to be expected if the membrane cholesterol mole fractions in Szabo membranes were about half of that of our membranes. I view of the Pagano et al. (1972) results and in view of the differences in dipole potential changes obtained by Szabo and us the cholesterol content in our membranes appears to be closely related to that of the membrane forming solution

Baumann & Mueller (1974) have reported an S-shape time course for the alamethicin-induced current relaxation. An S-shape time course has also been found by Boheim (1978), but it disappeared after high conductance was induced whereupon the time course became exponential. Our results in GMO and GMO-cholesterol membranes indicate that delay is always present in the current relaxations and takes place in the μs range

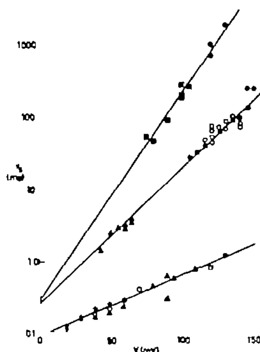


Fig 4 Plot of vs. V for different mole fractions of cholesterol in GMO membranes and different alamethicin concentrations. The lowest curve is for pure GMO membranes the middle curve is for 80%GMO–20%Cholesterol and the upper curve is for 50%GMO–50%Cholesterol. Both on and off relaxations are represented The different alamethicin concentrations are =33 ng/ml □=100 ng/ O=200 ng/ml =270 ng/ml, ▲=300 ng/ml =400 ng/ml =600 ng/ml, ●=900 ng/ml and ■=1600 ng/ml Note that depends on potential and cholesterol concentration, but not on alamethicin concentration. [NaCl]= 1 *M*

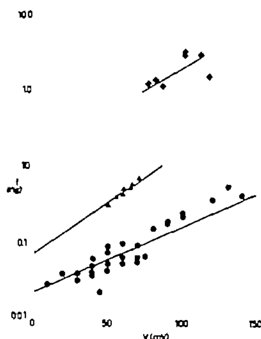


Fig 5 Plot of for different mole fractions of cholesterol in GMO membranes. The lowest curve is for pure GMO the middle curve for 80%GMO–20%Cholesterol, and the upper curve for 50%GMO–50%Cholesterol. [NaCl]=1 *M* Two or more concentrations of alamethicin are represented for each cholesterol mole fraction.

served dependence of τ_f and τ on voltage and temperature with the results of the analysis of long-lasting single channels Such a phenomenological comparison indicates that τ correlates well with the lifetime of single channels, and is taken to be due to the change in the number of channels present The fast relaxation process, on the other hand appears to arise from a shift in the probability distribution of the different conductance levels as the potential is changed τ_f *correlates well with the lifetime of the* different conductance levels

We have made such a correlation for GMO-Chol membranes containing a cholesterol mole fraction of 0.5 Fig. 6 shows a record of conductance fluctuations arising from a single alamethicin channel at an applied voltage of 190 mV It is apparent that the mean life-time of a single pore is on the same order as τ (see Fig 4) and that the mean half-life of the different conductance levels (bottom of Fig. 6) is on the same order as τ_f (see Fig. 5) Therefore in terms of the single alamethicin channel cholesterol increases the channel lifetime increases the dependence of channel lifetime on potential and increases the mean half-life of the different conductance levels.

As stated above the change in the steady-state alamethicin-induced conductance promoted by cholesterol could be accounted for by a decrease in the alamethicin adsorption coefficient an increase in membrane viscosity an increase in the membrane dipole potential or by changes in all these parameters simultaneously Taken together the steady-state data and the kinetic data allow us to

Changes in dipole potential can affect the alamethicin gating since we can view the alamethicin molecules as dipoles moving in an electric field the positive end of which is pushed into the membrane when the field is applied. Addition of cholesterol will increase the height of the energy barrier across which the positive end of the alamethicin molecules must move Yantorno et al (1977) have calculated the dipole moment of alamethicin to be of the order 67 Debyes.

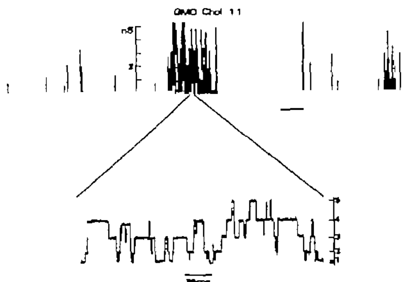


Fig 6 Current bursts due to single channels of alamethicin The expanded time scale below shows the different conductance levels more clearly and the scale on the right marks off the different levels. I this particular record up to six conductance levels can be seen 50%GMO–50%Cholesterol membrane. [Ala]=250 ng/ml, [NaCl]=1 *M* Applied potential, 190 mV Temperature: 22±1°C.

discuss in some detail each of these possibilities. Thus, the 3-fold change in the τ at zero potential ([illegible], see Table 2) is consistent with a change in the membrane viscosity but it would not explain why cholesterol increases the voltage dependence of [illegible]. The shift in the log G vs V curve (Fig. 1) is also difficult to explain in terms of fluidity since we would expect that changes in membrane viscosity would slow down both the rates of channel formation (μ) and channel closing (λ). Since the steady state conductance is given by Eisenberg et al. (1973)

$$G=\frac{\mu}{\lambda}$$

one would expect no change in the total number of open channels. In other words a change in membrane viscosity will change the activation energy barriers for channel opening and closing, but will not change the relative energies of the open and closed states

Either an increase in dipole potential or a change in the alamethicin adsorption coefficient may explain the change in g (Table 1) At present, we cannot discard either of these possibilities since we do not have independent measurements of the alamethicin partition coefficients as a function of cholesterol concentration. However if we assume that the change in g_+ is primarily due to a dipole potential change then we are forced to locate the open configuration somewhere in the middle of the membrane Taking into account this consideration together with the voltage dependence of the alamethicin-induced conductance we have calculated that the change in dipole potential felt by alamethicin in a 50%GMO–50%Chol membrane relative to a GMO membrane is about 80 mV This value is in good agreement with the value obtained using lipophilic ions as probes of changes in dipole potential (Donovan 1979)

Membrane composition and possibl modes of pore formation

The high power dependence of the conductance on the alamethicin concentration, and the multi-state

Table 2. *Alamethicin parameters for membran s of different composition*

Membrane	[illegible] (ms)	α^*	$\alpha^{\dagger}_{\tau_{s}}$	α'
GMO	0 09	4 4	0.51	3 9
80%GMO–20%Chol	0 25	4 1	1 1	3 0
50%GMO–50%Chol	0.27	4 1	1.60	2.5

Calculated from steady-state conductance data.
Defined according to the equation [illegible] = [illegible] exp ($\alpha\tau$ [illegible] V/kT).
Calculated according to [illegible]

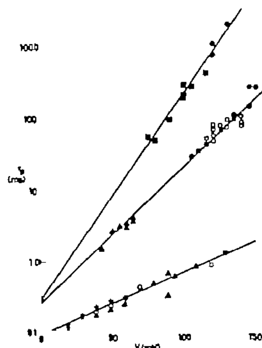
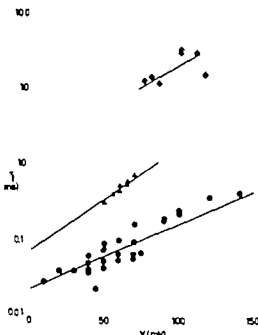


Fig 4 Plot of τ vs. V for different mole fractions of cholesterol in GMO membranes and different alamethicin concentrations. The lowest curve is for pure GMO membranes, the middle curve is for 80%GMO–20%Cholesterol and the upper curve is for 50%GMO–50%Cholesterol. Both on and off relaxations are represented The different hicin concentrations are =33 ng/ml □=100 ng/ ○=200 ng/ml, =770 ng/ml ▲=300 ng/ml =400 ng/ml =600 ng/ml, ●=900 ng/ml and ■=1 600 ng/ml. Note that depends on potential and cholesterol concentr-on, but not on alamethicin concentration. [NaCl]= 1 M



F g 5 Plot of for different mole fractions of cholesterol in GMO membranes. The lowest curve is for pure GMO the middle curve for 80%GMO–20%Cholesterol and the upper curve for 50%GMO–50%Cholesterol. [NaCl]=1 M Two or more concentrations of alamethicin are represent ed for each cholesterol mole fraction

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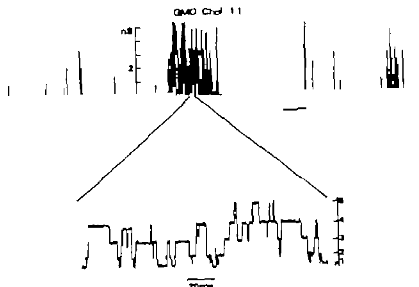


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discuss in some detail each of these possibilities Thus, the 3-fold change in the τ at zero potential (τ_{o} see Table 2) is consistent with a change in the membrane viscosity but it would not explain why cholesterol increases the voltage dependence of τ_{r}. The shift in the log G vs V curve (Fig. 1) is also difficult to explain in terms of fluidity since we would expect that changes in membrane viscosity would slow down both the rates of channel formation (μ) and channel closing (λ) Since the steady state conductance is given by Eisenberg et al (1973)

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Membran composition and possible modes of pore form tion

The high power dependence of the conductance on the alamethicin concentration, and the multi-state

Table ... *Alamethicin parameters for membranes of different composition*

Membrane	(ms)	α^a	α^b	α_d^c
GMO	0.09	4 4	0.51	3.9
80%GMO–20%Chol	0.25	4 1	1 1	3.0
50%GMO–50%Chol	0 27	4 1	1.68	[illegible]5

Calculated from steady-state conductance data.
Defined according to the equation [illegible] exp (α [illegible] V
Calculated according to [illegible]

character of the single alamethicin channels (Fig. 6) suggest that each channel is formed by association of several monomers of alamethicin However there is not a unique model describing the underlying molecular processes involved in the conductance transitions. Hall (1975) and Gordon & Haydon (1976) have proposed that the different conductance levels arise as a consequence of "conformational" changes of an oligomer containing a fixed number of monomers Baumann & Mueller (1974) Boheim (1974) and Boheim & Kolb (1978) on the other hand have proposed the existence of a pore that varies in diameter by uptake and release of monomers

Boheim & Kolb (1978) have argued strongly against the models of Hall (1975) Gordon and Haydon (1976) Their main line of argument is based on the alamethicin concentration dependence of τ in phosphatidylcholine alamethicin-treated membranes. They found that τ is well described by the equation

$$\tau = k[Ala]^{3} \exp(2.6eV/kT) \qquad (2)$$

where k is a proportionality constant This concentration dependence suggests that the pore does not contain a fixed number of monomers

Our results in GMO and GMO-Chol membranes [indi]cate that τ is independent of alamethicin con[cen]tration and that the voltage dependence of τ_e, regardless of the cholesterol mole fraction, is always less than that found in PC membranes by Boheim & Kolb (1978) The difference could be ascribed to differences in the lipid composition (GMO vs PC) or to the presence of solvent in the PC membranes However preliminary experiments performed by us using PE membranes formed by apposition of two monolayers indicate that is strongly dependent on alamethicin concentration in this type of bilayer as well. Apparently then, the molecular mechanisms which give rise to the conductance transitions are different in phospholipids than in GMO membranes

CONCLUSION

We have shown in this paper that the alamethicin-induced voltage-dependent permeability can be modified to a great extent by the nature of the lipid. In the following, we state the main results and conclusions which are presented in this paper (*a*) The voltage necessary to open a given number of alamethicin channels is increased by increasing the mole fraction of cholesterol in GMO membranes If the alamethicin channel opens by the process of orientation of the alamethicin molecular dipole in the electric field the above result can be understood in terms of an increase of the membrane dipole potential induced by cholesterol The shift to the right of the log G-V curve induced by cholesterol can also be explained in terms of changes in the amount of alamethicin adsorbed but not by changes in membrane viscosity (*b*) In many channel membranes, both the on and the off alamethicin-induced kinetic are altered by cholesterol Cholesterol slows down the alamethicin-induced kinetic and modified its voltage-dependence In terms of the single alamethicin channel the results can be interpreted as an increase in the half life of the channel and a change of the equilibrium position of the closed configuration of the alamethicin channel respectively (*c*) Comparison of the kinetic processes describing channel decay in GMO and phospholipid bilayer membranes suggests that the mechanism of formation of the alamethicin channel is different in these two types of membranes

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Effects of lipid structure on the kinetics of carrier mediated ion transport

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BENZ, R. CROS D JANKO K. LÄUGER, P & STARK, G Effects of lipid structure on the kinetics of carrier-mediated ion transport. Acta Physiol Scand 1980 Suppl 481 47–52. Department of Biology University of Konstanz, Germany

The mechanism of alkali-ion transport mediated by valinomycin (or similar macrocyclic carriers) may be studied using artificial planar bilayer membranes The rate constants of the single transport steps (association and dissociation of the ion-carrier complex, translocation of free and complexed carrier) can be determined from electrical relaxation experiments The turnover number of valinomycin which may be calculated from the rate constants is found to be 10^4 10^5 s Carriers of the valinomycin-type offer the possibility of studying the relationship between membrane structure and transport kinetics. Increasing the chain-length of the lipid strongly reduces the translocation rate constants of the free and the loaded carrier and also (in the case of lecithin membranes) the association rate constant. Increasing the number of double bonds in the fatty-acid residue of the lipid leads to an increase of the translocation rate constants. These effects are discussed in terms of microviscosity of the membrane Addition of cholesterol to monoglyceride membranes seems to affect both the microviscosity and the dipolar potential at the membrane-solution interface

Certain antibiotics such as valinomycin monactin, or enniatin B have been shown to act as mobile ion carriers which facilitate passive ion transport through lipid bilayer membranes (Ovchinnikov et al 1974). These compounds form complexes with alkali ions in which the ion is surrounded by a cage of oxygen atoms. In the past years artificial planar bilayer membranes have been extensively used for studying the mechanism of carrier-mediated ion transport (Mueller & Rudin 1967 Andreoli et al 1967 Lev & Buzhinsky 1967 Eisenman et al 1968 Liberman & Topaly 1968 Stark & Benz 1971) From the dependence of membrane conductance on the concentration of potassium and valinomycin it has been concluded that potassium is transported as a 1 1 complex between ion and carrier (Stark & Benz 1971). In the presence of the carrier ion transport through the membrane-solution interface may occur in either of two ways (Benz et al 1976). The ion-carrier complex may form already in the aqueous solution and may then cross the interface ("solution-complexation mechanism). Alternatively a hydrated ion from the aqueous phase may react in the interface with a carrier molecule located in the lipid ("interfacial-complexation mechanism") The complex then moves to the other interface where the ion is released to the aqueous solution. If the first mechanism would be operative, diffusion polarization should occur at high current densities i.e. the carrier should be depleted in the aqueous phase on one side and accumulated at the other Such a diffusion polarization is never observed in the presence of valinomycin and potassium and this gives strong evidence that in this system the interfacial-complexation mechanism is operative (Stark & Benz 1971) The same conclusion holds true for other cation carriers such as monactin (Stark & Benz 1971) or enniatin B (Benz 1978) An example for the solution-complexation mechanism has been found however in the case of a synthetic prolin-containing analog of valinomycin

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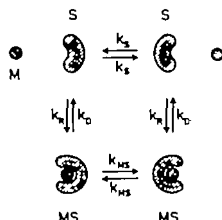


Fig 1 Transport of ion M mediated by a carrier S.

(Ting-Beall et al 1974 Benz et al 1976 Andersen et al. 1977)

Electrical relaxation methods for the study of carrier kinetics

According to the interfacial-complexation mechanism the overall transport process occurs in four distinct steps (Fig. 1) (i) association of ion M and carrier S at the interface (rate constant k_R) (ii) translocation of the complex MS to the other interface (rate constant k_{MS}) (iii) dissociation of the (rate constant k_D) and (iv) back-transport carrier S (rate constant k_s) For the study the kinetics of this reaction and the determination of the single rate-constants fast relaxation techniques have to be used Two different electrical relaxation methods have been applied to carrier systems (Fig.) In the voltage-jump current-relaxation method the voltage across the membrane is quickly changed and the approach of the membrane current towards a stationary value is followed (Stark et al. 1971) A somewhat better time resolution (down to about 200 ns) may be achieved with the so-called charge-pulse technique (Benz & Läuger 1976) In this method the membrane capacitance is charged up to an initial voltage of say 10 mV by a brief current-pulse (Fig. 2) Thereafter the external circuit is switched to virtually infinite resistance and the decay of membrane voltage (caused by ion conduction processes in the membrane) is followed

Determination of the rate onstant of the individual transport steps

The analysis of the carrier model represented in Fig 1 yields the following expression for the time course of membrane voltage V in the charge-pulse experiment

$$V(t)=V [a\ \exp(-t/\tau\ )+a\ \exp(-t/\tau\ ) +a\ \exp(-t/\tau_3)] \quad (1)$$

$$a\ +a_2+a_3=1 \quad (2)$$

where V is the initial voltage The relaxation times τ τ τ and the relaxation amplitudes a a_2, a_3 are complicated functions of the rate constants (Benz & Läuger 1976) Under favourable experimental conditions where all three relaxation processes can be resolved the rate constants k_R k_D, k_{MS} and k_s (Fig 1) can be determined from records of V(t) A similar analysis is possible for voltage-jump current relaxation experiments (Stark et al. 1971)

Rate constants for a number of cation-carrier systems have been evaluated in the last years by these methods. As a specific example we consider the results obtained for valinomycin/Rb in a monoolein/*n*-decane membrane (25°C, 1 *M* RbCl) (Benz & Läuger 1976)

$$k_R= 3\times10^{5}\ M\ \ s$$
$$k\ = 2\times10^{5}\ s$$
$$k_{MS}=3\times10^{5}\ s$$
$$k\ = 4\times10^{4}\ s$$

At one-molar concentration of the transported ion ($c_M=1$ *M*) the rate constants of association ($c_M k_R$) dissociation (k_D) and translocation of the loaded carrier (k_{MS}) are approximately equal (2–3×10⁵ s⁻¹) The rate-determining step in this system is the back-transport of the free carrier (k =4×10⁴ s) $k_{MS}=3\times10^5$ s is the frequency of jumps of an

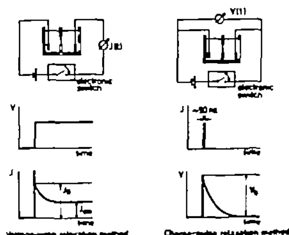


Fig 2 Electrical relaxation method for the study of ion transport kinetics in planar bilayer membranes.

Table 1 *Rate constants of valinomycin-mediated Rb transport through membranes made from phosphatidylcholines (PC) or α-monoglycerides (G) with different length of the mono-unsaturated fatty acid residue palmitoleoyl (16 1 1) oleoyl (18 1) Δ -eicosenoyl (20 1) erucoyl (22 1) and nervonoyl (22 1) (Bent et al 1973 Bent et al 1977)*

The solvent for membrane formation was n-decane; T=25°C. Data for monoglycerides have been obtained from charge-pulse experiments, data for phosphatidylcholines from voltage-jump or (denoted by an asterisk) from charge pulse experiments

Lipid	$k_R/10^4 M^{-1}$	$k_D/10^4 s^{-1}$	$k_{MS}/10^4$	$k_S/10^4$
di-(16:1)-PC	4.5(8.2*)	45	9 1	1.3(2.2*)
di-(18 1)-PC	2 1	–	–	1 1
di-(22 1)-PC	0.26	–	–	0 4
di-(24 1)-PC	0.026		–	0 077
(16 1)-G	43	13	74	8.5
(18 1)-G	37	24	27	3.5
(20 1)-G	23	12	10	1.8
(22 1)-G	24	13	7	1 1

ion-carrier complex from one interface to the other the reciprocal value $1/k_{MS}=3 \mu s$ is the average time required for translocation. This time may be compared with the diffusion time $\tau=d^2/2D$ of a spherical particle of the size of the carrier (radius ≈0 7 nm) across the same distance (membrane thickness d≈5 nm) in water (diffusion coefficient $D=3\times10^{-6}$ cm² s) which is about 0 04 μs From the rate constants, another important quantity may be calculated the maximum turnover rate f of the carrier f is defined as the limiting transport rate which is approached under short-circuit conditions for infinite ion concentration on the cis-side and zero ion concentration on the trans-side. f may be calculated from the known values of the rate-constants (Läuger 1972)

$$f=\left(\frac{1}{k_{MS}}+\frac{1}{k_S}+\frac{1}{k_D}\right) \quad (3)$$

In the above example, f is about 3×10^4 s The high efficiency of valinomycin as an ion carrier mainly results from this high turnove rate whereas the binding constant for the ion $k_R/k_D=1.5$ M is rather low

Influence of m mbra e structure on th rat constant

(a) *Vari tion of chain-l ngth of the lipid* Carrier systems of the valinomycin type offer the possibility of studying the relationship between membrane structure and transport rates. The kinetics of valinomycin-mediated Rb transport in membranes made from different phosphatidylcholines (lecithins) has been studied with voltage jump experiments (Benz et al 1973) The phosphatidylcholines used in these experiments contained mono-unsaturated fatty acids of different chain-length (C_{16} to C_{24}) It is seen from Table 1 that increasing the chain-length from C_{16} to C_{24} resulted in a drastic decrease of the association rate-constant k_R and the translocation rate constant k_S of free carrier (for technical reasons the two other rate constants could not be determined in all cases) k_R decreased by a factor of about 200 and k_S by a factor of about 20.

The decrease of the translocation rate constant k_S of the free carrier with chain length presumably reflects an increase of microviscosity of the membrane For the surprisingly strong chain-length dependence of the association rate constant k_R at least two possible explanations have to be considered One possibility consists in assuming that the uncomplexed carrier is present in the membrane in a conformational state which differs significantly from the compact conformation of the carrier i the complexed form In this case complexation with the ion would require a major conformational change the rate of which should depend on the microviscosity of the environment. Another possibility is that the uncomplexed carrier is located more towards the hydrocarbon interior of the membrane An ion entering the carrier from the aqueous solution then has to pass through the zone of the polar head-groups of the lipid. As the average location of the carrier with respect to the interface may depend

Table 2. *Rate constants of valinomycin-mediated Rb transport through membranes made from α-monoglycerides (G) differing in the number of double-bonds in the fatty-acid residue oleoyl (18 1) linoleoyl (18 2) linolenoyl (18 3) Δ -eicosenoyl (20 1) Δ -eicosadienoyl (20 2) Δ ¹⁴ ¹¹-eicosatrienoyl (?0 3) and arachidonoyl (20 4) (Ben_ et al 1977)*

The solvent for membrane formation was *n*-decane $T=25°C$

Lipid	$k_R/10^4 M$ s	$k_D/10^4$ s	$k_{MS}/10^4$ s^{-1}	$k_S/10^4$ s
(18 1)-G	37	24	27	3.5
(18 2)-G	67	13	143	6.2
(18 3)-G	74	8	⌊50	9 6
(20 1)-G	23	12	10	1.8
(20 2)-G	34	9	39	3.2
(20 3)-G	34	5	136	9 4
(20 4)-G	42	3	240	12

on the nature of the hydrocarbon chain a variation of chain length could result in a change of the energy barrier which has to be surmounted by the ion and therefore in a change of association rate Possibly both effects contribute to the variation of k_R with lipid chain-length

In a second series of experiments membranes made from monoglycerides differing in the fatty acid residue were studied (Benz et al 1977) In this case the variation of k_R with chain length was small, but again a pronounced decrease of k also of k_{MS}) with increasing chain length was ind (Table 1)

(b) Variation of the number of double bonds Kinetic parameters for valinomycin-mediated Rb transport in membranes made from monoglycerides with varying degree of unsaturation (Benz et al 1977) are given in Table 2. Again k_R and k_D are not much sensitive to a variation in the number of m of double bonds. On the other hand the translocation rate constant k_{MS} (and to a lesser degree also k_S) strongly increases with n the change of k_{MS} being 4-fold between $n=1$ and $n=4$ This variation of k_{MS} presumably results (at least in part) from an increased disorder and decreased microviscosity in membranes made from highly unsaturated monoglycerides. Another effect which may influence k_{MS} is the increase of dielectric constant of the membrane with increasing unsaturation Such an increase of dielectric constant would reduce the energy barrier for the translocation of the charged complex

(c) Effect of polar head-group A comparison of the data for di-(16 1)-PC and (16 1)-G in Table 1 may serve to illustrate the effects of the polar head group. Both lipids have identical fatty acid chains (palmitoleic acid) but differ in their polar residues Dipalmitoleoyl phosphatidylcholine and glycerol monopalmitoleate differ considerably in all four rate constants The most striking difference occurs in the value of the stability constant $K_S=k_R/k_D$ of the complex, K being about 20 times larger in the case of the monoglyceride as compared with the phosphatidylcholine Recently Hladky & Haydon (1973) have studied the stationary membrane conductance λ_0 of lecithin and monoglyceride membranes in the presence of a cationic carrier system (nonactin/K) and have presented arguments that the large difference in λ_0 results from the existence of a dipolar potential in the membrane solution interface which makes the interior of a lecithin membrane more positive than the interior of a monoglyceride membrane The finding that K_S is larger in a monoglyceride membrane than in a phosphatidyl choline membrane is consistent with this picture However it is seen from Table 1 that also the two translocation rate constants k_{MS} and k_S differ considerably in membranes made from the two lipids The influence on k_S may be discussed on the basis of a higher fluidity of the monoglyceride membranes compared with phosphatidylcholine membranes. Furthermore it is not clear to what extent a carrier can be influenced by an existing dipole potential. The valinomycin-cation complex has a diameter of 1.5 nm and a height of 1.2 nm a molecule of this size necessarily creates a large local perturbation of the structure of the dipole layer

(d) Incorporation of cholesterol Incorporation of cholesterol into a glycerolmonooleate membrane

Table 3 *Influence of cholesterol on the rate constants of valinomycin-mediated Rb transport (Benz & Cros 1978)*

The membranes have been made from mixtures of glycerolmonooleate and cholesterol in *n*-hexadecane (T=25°C). The mole fraction of cholesterol (referred to total lipid) is given in the first column

x	$k_R/10^4$ M^{-1}	$k_D/10^4$ s	$k_{MS}/10^4$ s	$k_S/10^4$ s
0	15	9	23	3 0
0.17	13	11	19	2.7
0 33	8.3	12	14	1 7
0 50	6.2	10	7 1	1 0
0 67	3.0	1	4.2	0.62
0.80	2.0	11	2 8	0.41

(Benz & Cros 1978) reduces k_R k_{MS} and k_S up to about 8-fold (Table 3) In these experiments *n*-hexadecane has been used as solvent for membrane formation In this case the membrane thickness as determined by capacitance measurements is independent of cholesterol content (this is no longer true for membranes containing *n*-decane (Benz et al. 1977 Hanai et al 1965))

Cholesterol may affect the structure of the membrane and the kinetics of carrier transport in different ways (Hladky & Haydon 1973 Szabo 1974 1976 McLaughlin 1976) By interacting with the hydrocarbon chains of the lipid molecules cholesterol may reduce the fluidity of the membrane and thereby decrease the translocation rate of the carrier On the other hand there is experimental evidence that cholesterol changes the dipolar potential of the membrane so that the interior of the membrane becomes more positive (Hladky & Haydon 1973 Szabo 1974 1976 McLaughlin 1976) Depending on the location of the adsorbed ion-carrier complex with respect to the plane of the dipoles a change in the dipolar potential may affect the stability constant K_s of the complex as well as the translocation rate constant k_{MS} The observation that the effect of cholesterol on k_{MS} is about the same as on k_s seems to indicate that the translocation rate constant of the charged complex is not significantly influenced by the dipole potential On the other hand it is seen from Table 3 that the stability constant $K_s = k_R/k_D$ decreases about 7 fold if the mole fraction x of cholesterol is increased from 0 to 0 8 Both findings taken together may be interpreted by the assumption that the charged complex is located on the hydrocarbon side of the dipole layer This interpretation has to be considered as tentative however in view of the difficulties discussed above in the quantitative application of the dipolar-layer model to carrier mediated ion transport

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THE EFFECTS OF ANTRAL DISTENSION ON GASTRIC ACID SECRETION AND ON THE RELEASE OF GASTROINTESTINAL HORMONES IN MAN

By
Ivl Mai Schöön

GÖTEBORG 1980

ACTA PHYSIOLOGICA SCANDINAVICA

SUPPLEMENTUM 482

From the Department of Surgery II Sahlgren s Hospital University of Göteborg Göteborg Sweden

THE EFFECTS OF ANTRAL DISTENSION ON GASTRIC ACID SECRETION AND ON GASTROINTESTINAL HORMONES IN MAN

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Ivi-Mai Schöön

Göteborg 1980

This summary is based mainly on studies reported in the following papers:

I Evidence for a defective inhibition of pentagastrin-stimulated gastric acid secretion by antral distension in the duodenal ulcer patient
Schöön I -H Bergegårdh S Grötzinger U & Olbe L
Gastroenterology 75:363-367 1978

II A study of the effect of antral distension on gastric acid secretion in man
Schöön I -H Lundqvist G Rehfeld J & Olbe L
Digestion accepted for publ

III The effects of beta adrenergic receptor blockade on the inhibition of gastric acid secretion and the release of pancreatic polypeptide by antral distension in healthy subjects
Schöön I -H Lundborg P Schwartz T W & Olbe L
Scand J Gastroent accepted for publ

IV The effect of antral distension in healthy subjects on betazole stimulated gastric acid secretion and the plasma concentration of immunoreactive neurotensin
Schöön I -H Bloom S R & Olbe L
Scand J Gastroent accepted for publ

V The effect of antral distension on the endocrine pancreas in man
Schöön I -H Sjöström L Holst J J Schwartz T W & Olbe L
Scand J Gastroent accepted for publ

These papers are referred to in the text by their Roman numeral

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ACTA PHYSIOLOGICA SCANDINAVICA

SUPPLEMENTUM 482

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SITY OF GÖTEBORG GÖTEBORG SWEDEN

Abstract

SCHÖÖN I -M *The effects of antral distension on gastric acid secretion and on the release of gastrointestinal hormones*

Antral distension in pouch dogs has been found to stimulate gastric acid secretion both via gastrin release and ia reflex pathways to the parietal cell area In an acid intragastric milieu additional inhibitory action has been presented in the dog In duodenal ulcer patients graded balloon distension has been shown to stimulate gastric acid secretion ithout any release of gastrin and independent of intragastric pH In healthy subject significant decrease of gastric acid secretion was evoked by antral distension Gastric distension in man has resulted in stimulation of exocrine pancreatic secretion and release of pancreatic polypeptide

In the present study antral distension by 150 cm^3 balloon volume had no ffect on submaximal pentagastrin-stimulated gastric acid secretion in duodenal ulcer patients whereas the secretion was inhibited by 20 % in healthy subjects (±30) The inhibi tion of gastri acid secretion by antral distension persisted during neutralization of gastric contents and did not result in an increase f somatostatin concentration in portal venous blood or in gastric contents Antral distension inhibited pentagastrin- and betazole-stimulated gast ic acid secretion to the same extent which ex ludes that the gastrointestinal peptides secretin cholecystokinin and bulbogastrone mediat the inhib tion of gastri acid secretion During antral distension the concentration of neurotensin potent inhibitor of gastric acid secretion was unchanged in portal and peripheral venous blood in non-ulcer patients and in duodenal ulcer patients Antral distension released pancreatic glucagon and pancreatic polypeptide and VIP to portal venous blood in both non-ulcer and duodenal ulcer patient suppor ting the concept f gastro-pancreatic reflex to the endocrine part of pancreas The results however indicate that no gastrointestinal peptide with established acid inhibitory property seems to mediate the inhibitory ffect f antral distension on gastric acid secretion

The rapid onset of the inhibition f gastri acid produced by antral distension and the immediate reversal of this inhibition mimicks the pattern of reflex mechanism The inhibitory ffect of antral distension remained however fter beta$_1$ and beta$_{1+2}$ receptor blockade and fter intramuscular injection of the dopaminergi antagoni t metoclopramide An inhibitory reflex acting on ganglioni level is to some extent supported by preliminary data showing that antral distension in healthy subject inhib ted vagally timulated gastric acid secretion more ffi ienti than pentagastrin-stimulated gastric aci secretion

Key-words antral distension betazole duodenal ulcer gastric acid secretion gastrin gastro-pancreatic reflex metoclopramide metoprolol pentagastrin neurotensin pancreatic glucagon pancreatic polypeptide propranolol somatostatin

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"When you can measure what you are speaking about and express it in numbers you know something about it but when you cannot measure it when you cannot express it in numbers, your knowledge is f meagre and unsatisfactory kind"

lord Kel in

Introduction

The study of gastric secretion is much younger than that of gastric motility the latter discribed as "coction was already known to Hippocrates 460 370 B C The classical experiments of Reaumur (101) and Spallanzani (111) demonstrated that gastric digestion involved a chemical process In 1833 Beaumont published his observations on a patient Alexis St Martin who was left with a stomach fistula after a severe gunshot wound By applying mechanical irritation such as the pressure f a thermometer to the internal coat of the stomach he observed that distinctly acid fluid wa secreted (9) From this report Blondlot (16) and Bassow (6) conceived the idea f artificially producing gastric fistula dogs but they failed to excite gastric secretion by using mechanical irritation (friction) In these early investi gations the sit of stimul tion (antral or fundic mucosa) is not distinctly described nor is the type f mechanical irritation Mechanical scratching may have failed to stimulate acid secretion by damaging the mucosa The introduction by Klemensiewic (72) of the pouch method (in this instance in the pyloric region) suggested to Heidenhain the construction of a fundus pouch (55) Heidenhain appears to have been the first person to use balloon distension to stimulate gastric acid secretion in pouch dogs His conclusion was that local timul tion had only local ffect Contradictory to thi Pavlov (94) suggested that the mechanical stimulation of gastric acid secretion observed by earlier invest gators may be of long reflex origin Zelinoy and Savitsch working on dogs with pyloric pouches and gastric fistulas found that mechanical irrit tion of the pyloric mucosa augmented secretion in the fundus even after the "pyloric ner ves had been divided (136) In 1925 Lim Ivy and McCarthy re iewed the lder xperiments on the timulation of gastric secretion by local (mechanical and chemical) stimulants Lim et al performed their own investigations carried out in dogs with

some observations on normal man included (80) As far as mecha nical stimulation was concerned they concluded that 1) "mecha nical distension of the stomach stimulates gastric secretion in man and in the dog in the latter with or without intact vagus nerves and 2) the stimulation of gastric secretion by mechani cal distension is inhibited by the hypodermic administration of 1 mg atropine and by the introduction of fat into the small in- testine In 1974 Debas and co-workers used a pouch dog very similar to that of Zelinoy and Savitsch but they had totally different resources available to evaluate the results of antral distension (29) In their preparation of the innervated antral pouch gastric fistula and Heidenhain pouch distension of the antral pouch with sodium bicarbonate effectively stimulated the release of gastrin from the antrum and secretion of acid from both the gastric fistula and the Heidenhain pouch When the ant rum was distended with acid both the serum gastrin and Heiden- hain pouch responses were completely suppressed However the acid response from the body of the stomach with a gastric fistu la was not abolished Total denervation of the antrum abolished the acid secretion response of the stomach body to distension of the antral pouch The conclusion was that antral distension evoked acid secretion by release of gastrin and by a vagal sti mulatory pyloro-oxyntic reflex The effect of distension on ant ral G-cells appears to be mediated by intrinsic and vago-vagal refle es (31) However the type of transmission in these re- flexes is uncertain Small doses of atropine (less than 25 $\mu g/kg$) enhanced the gastrin response to sham feeding (39 113) insulin (37) and meal stimulation (128) in man Gastrin release evoked by bombesin was inhibited by cholinergic drugs in dogs (115) The results indicate: 1) cholinergic inhibition of gastrin re lease 2) reflex release of gastrin by a non-cholinergic other wise unknown transmission mechanism

In the experiments of Yamogoshi and Debas on innervated antral pouch-dogs antral distension with acid resulted in the inhibi tion of pentagastrin-stimulated gastric acid secretion (135)

Distension of the fundic region of the stomach in the dog evokes gastric acid secretion apparently by stimulating a cholinergic refle both of long vago-vagal and short intramural type (49

50 99 81) These reflexes have been characterised as oxynto-oxyntic (29) An oxynto-pyloric reflex pathway was suggested by experiments in which distension of a Pavlov pouch caused a release of gastrin from an innervated alkaline antral pouch (30)

In man the results of investigations on "mechanical stimulation of gastric acid secretion have revealed some important differences compared to the results in dogs

In 1901 Schûle failed to stimulate gastric acid secretion by mechanical stimulation in man using vigurous touching of the stomach wall with a soft rubber tube (106) Lim and co-workers performed whole stomach distension in man with rubber balloons (80) and three out of five men showed a definit stimulation of gastric acid secretion By distending the stomach of healthy subjects with 500 ml of a chemically inert test meal at pH 5 0 in healthy subjects Richardson and co-workers were able to conclude that distension is an important stimulant of the acid-secretory response to a meal and that stimulation was not mediated by gastrin release (102) Schrumpf and Stadaas (105) in their studies f balloon distension of the whole stomach in healthy subjects also failed to evoke any increase of plasma gastrin levels The same results were obtained by Soares et al (110)

Separate fundic distension in healthy subjects as well a in duodenal ulcer patients resulted in an increased gastric acid secretion with increasing distension volume up to 600 800 ml (51) The highest acid output amounted to just above 50 per cent of the maximal secretory response to pentagastrin The stimula tion by fundic distension was blocked by i v tropine and par tially inhibited by proximal gastric vagotomy (52) Fundic distension was thus considered to stimulate gastric acid secretion by means of an atropine-sensitive reflex mechanism involving both short intramural and long vago-vagal pathways Plasma gas trin lev ls were unchanged during separate fundic distension in duodenal ulcer patients (52) even when gastric contents were neutralised

Previous studies have shown evidence that antral distension

healthy subjects does not increase basal acid secretion irre spective of whether the pH of the gastric contents is low (about 2 5) or high (above 7) (12 10) In fact a slight but statisti cally significant decrease of acid secretion was observed in healthy subjects Similar studies in duodenal patients however show evidence that separate graded antral distension increases acid secretion in a stepwise fashion At 150 cm^3 balloon volume the secretory response amounted to about 35 % of the maximal acid response to pentagastrin (12 11) Basal plasma gastrin le vel remained unchanged by antral distension in these duodenal ulcer patients even when the intragastric milieu was alkaline (11) The acid response in duodenal ulcer patients was suggested to be mediated via a pyloro-oxyntic reflex (11) quite opposite to the findings in dogs where the gastrin mechanism predominated (29) A marked difference in the acid responses to the antral distension between duodenal ulcer patients and healthy subjects with stimulation occurring only in the ulcer patients was demonstrated (12)

Apparently antral distension in man affects gastric acid secre tion in two ways one stimulating and the other inhibiting gast ric acid secretion As regards the stimulatory effect this has been suggested to be mediated by means of a reflex activation As far as the inhibitory response is concerned the mechanism in- volved is obscure Previous studies show evidence that there are probably several nervous and/or neurohormonal factors which might directly or indirectly suppress gastric secretion For example vagal stimulation has been shown no result in increase of the gastric HCO_3 secretion (40) and in acid intragastric milieu such stimulation releases somatostatin known to inhibit gastric secretion (122 53) Moreover there are strong evidence that antral distension directly activates adrenergic neurones suppressing gastric motility (1) and that gastric distension might also stimulate the gastro-pancreatic reflex to the exocri ne part of the pancreas shown to involve both vagal and splanch- nic nerves (130 132) Antral distension has recently been shown to activate gastric receptors with slowly adapting charge recor ded in splanchnic nerve afferents (41) Both basal and penta gastrin-stimulated gastric acid secretion is inhibited by adre nergic agonists (28 45) without decreasing gastric blood flow

Adrenergic presynaptic inhibitory effects on gastrointestinal motility have also been demonstrated (125) It therefore appears possible that adrenergic reflex mechanisms evoked by antral dis tension might be involved in inhibition of gastric acid secretion

As far as the gastro-pancreatic reflex pathway is concerned the reflex to the exocrine part of pancreas has been established both in the dog and in man (14 82 131 129 130 132) Vagal reflex release of pancreatic polypeptide available from both exocrine and endocrine part of the pancreas (76) has been strongly suggested in man (107) However since the amount of this hormone as released by sham feeding is diminished and the response delayed after propranolol treatment a beta adrener gi component was indicated (3) A gastro-pancreatic reflex release of pancreatic glucagon a potent inhibitor of gastric acid secretion also known to be released by both cholinergi and adrenergi timulation (34) seems to be a neurohumoral possibility f inhibition of gastric acid secretion

Also a dopaminergic r flex inhibition may be considered because f intravenous infusion f dopamine inhibits both basal and pentagastrin-stimulated gast ic acid secretion in man in dog (123 24 124) and this inhibition is abolished by pretreatment ith metoclopramide (120)

Besides the abovementioned neurohormonal release purely hormonal inhibition of gastric acid secretion also has to be taken into account Thus secretin cholecystokinin bulbogastrone and *probably also neurotensin inhibit gastric acid secretion* when stimulated by pentagastrin However these hormones fail to inhibit acid secretion when h stamine is used for stimulation (47 44 67 57 118 2 22) Although the failure of antral distension to inhibit betazole-stimulated gastric acid secretion can reasonably exclude these gastrointestinal hormones as possible mediators f the antral-distension-released gastric-acid inhibition the demonstr tion of unchanged blood concentration of the individual hormones during antral distension will support such a statement

AIM OF THE PRESENT STUDIES

The central and local nervous and neurohormonal control of gastric acid secretion is in many respects still poorly understood Previous results on vagal stimulation and/or hormonal inhibition of gastric acid secretion are to a considerable extent controversial The divergent opinions met in the literature may often be explained by different experimental conditions incomparable patients or species examined and the use of unphysiological stimuli Moreover not until recently methods have become available to study the gastrointestinal hormones by determination of the fluctuations in their blood concentrations in response to different physiological stimulatory inhibitory effects

The principal aim of the present study was therefore:

1 To compare qualitatively and quantitatively the response of separate antral balloon distension on pentagastrin-stimulated gastric acid secretion in duodenal ulcer patients and healthy subjects To explore more in detail the mechanism that might be involved in these responses particularly as regards nervous and neurohormonal mechanisms

2 To study qualitatively and quantitatively the response of separate antral balloon distension on betazole stimulated gastric acid secretion in healthy subjects This investigation was considered of particular interest since inhibition of betazole stimul ted gastric acid secretion by antral distension would e clude secretin cholecystokinin and bulbogastrone as possible mediators

3 To determine the plasma concentration of somatostatin neurotensin and glucagon all potent inhibitors of gastric acid secretion in portal and peripheral venous blood and if possible also to analyse somatostatin in gastric contents In this context it was considered of interest also to assess the gastrin concentration in portal and peripheral venous blood for further evaluation of a possible release of somatostatin which should suppress the gastrin concentrations (122 53)

4 To evaluate whether β adrenergi reflex activation or a dopaminergic transmission might be a cause of gastric acid inhibition as evoked by antral distension

5 To study to what extent separate antral balloon distension

in healthy subjects might affect the endocrine part of the pancreas and the relationship of pancreatic glucagon insulin and glucose in peripheral venous blood in an attempt to shed further light on the gastro-pancreatic reflex mechanisms

METHODOLOGICAL CONSIDERATIONS

A *Determination of gastric acid secretion*

To check recovery constant perfusion of the stomach using 225 ml $(15\ min)^{-1}$ of a marker solution (8 mg phenol red per litre) water was implayed The nasogastric perfusion tube (Intramedic PE 160) was inserted into the stomach with its tip 5 to 10 cm below the cardia A high perfusion volume facilitated mixing of the marker and the gastric juice The gastric contents were continuously aspirated using a suction pump with intermittent negative pressure (~50 mmHg once/second) Due to continuous aspiration gastric distension was avoided It might be argued that the marker might to some extent be absorbed interfering with the quantitative estimations However such an error appears less likely The loss of phenol red through the canine gastri mucosa in a Heidenhain pouch during histamine stimulated secretion is in fact less than 5 per cent (17) and the abs rption of phenol red in the lumen of the human stomach is probably still lower and independent of the pH f the gastr c contents (58 64 95) Moreov r the phenol red concentration in 10 ml of each sample was determined spectrophotometrically at a wavelength of 565 µm after filtration (Millipore filter 1 2 µm) and alkalinization with 0 04 ml of 2 4 moles per litre of NaOH making any interference with various bile p gments minimal Assuming simi lar percentage loss of gastr c acid and phenol red vi the pylorus the total amount of secreted cid could be calculated from the recovered quantity of phenol red Quantitative estimation f duodeno-gastric reflux was not used in the present studies

Determination of the concentration of hydrogen ions was carried out by potentiometrical titration f 100 ml of the sample to pH 7 0 when water perfusion of the stomach was used In the experiments with alkaline buffer perfusion of the stomach (II) the perfusate consisted of phenol red in 1/15 M Sörensen s phosphate

buffer pH 8 3 (162 mosm l[1]) the perfusion rate still being 225 ml (15 min)[1] 100 ml of each sample was titrated to pH 9 5 The titration value of the sample minus the titration value of the infused amount of buffer solution per 15 min (titrated to pH 9 5) was expressed as acid output in mmol (15 min)[1]

B *Antral balloon distension*

A small thin-walled latex balloon firmly tied to a polyethylene tube of 3 mm diameter that was attached to a double lumen nasogastric tube (Salem sump no 14) in such a way that the latex balloon when insufflated was placed in front of the tip of the sump tube Loosely attached to these two tubes was a third thin polyethylene tube (for perfusion of the water or buffer solution of phenol red) which could be retracted with its tip placed in the groups region of the stomach

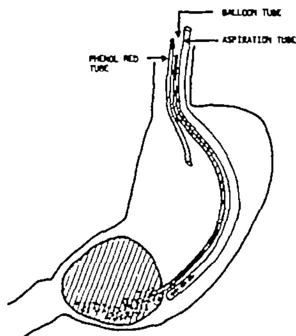


Fig 1 Schematic illustration of the arrangement of the nasogastric tubes

With the empty balloon wrinkled around the Salem sump tube the equipment could be passed through a nostril and placed in the stomach The position of the nasogastric tube was controlled by fluoroscopy of the X ray visible thred in the Salem tube After the determination of basal acid secretion during three to four 15 min periods the balloon was filled with 150 cm^3 water solu tion of a roentgen contrast medium (Gastrografin) (Fig 1) The intraballoon pressure was calculated to about 32 cm H_2O The position of the balloon was checked throughout the experiment by fluoroscopy The volume of 150 cm^3 was chosen to ensure a separate antral distension because at balloon volumes exceeding 150 cm^3 the balloon tended to bulge into the corpus region (12)

For anatomical reasons and to prevent sliding of the balloon in the laparotomy experiments this was filled with 150 cm^3 air The position of the balloon was then checked manually throughout the 30 min period of distension and in all experiments the volu me of contrast or air was carefully measured at deflation of the balloon The distension of the antrum using closed balloon system is considered to be predominantly isometri The balloon remained in place in the antrum as checked by fluoroscopy and no discomfort was observed in any patient during its distension

The advantage of balloon distension a compared with the more conventional fluid distension are obvious no chemical or osmo- tic influence on the di tended area can interf re with mechani cal stimulation The antrum has a complex autonomic innervation Most of the abdominal vagus and the splenchnic nerves consist of aff rent nerve fibres from the gastro-intestinal tract Singl fibre records from vagal fferents (93 60 61) reveal two dis- tinct cathegories of gastric mechanoreceptors slowly adapting mechanoreceptors and rapidly adapting mechanoreceptors with some chemoreceptor properties (62) and a sustained response is ob tained on a steady distension (93 60) Moreover passive dis- tension of an visceral organ and iso-metric contraction in the receptor area has been shown to activate slowly adapting mecha noreceptors which are considered to be functionally coupled i series (78) Gastric receptors with splenchnic nerve afferents give slowly adapting discharge to antral distension (41)

similar to the impulses in splanchnic nerve afferents from me chanoreceptors in the mesentery (91) There is evidence that antral distension in the cat elicits two different gastro-gastric reflex mechanisms suppressing the gastric smooth muscle activity These reflexes are mediated via the vagal and splanchnic nerves (1)

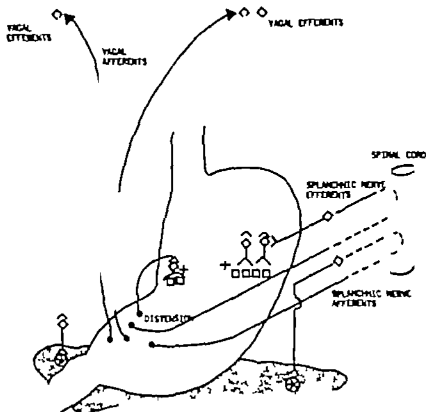


Fig 2 Schemati diagram of possibl Vagal and Splanchnic reflex mechanisms

As shown in *Fig 2* it appears that antral distension as applied in the present study could possibly activate several afferent neurones eliciting at least theoretically stimulatory and/or inhibitory effects on gastric acid secretion Thus equivocal and divergent results often obtained in previous studies of gastric function may partly be explained by the fact that different modes of mechanical timul tion have often been utilized

RESULTS AND COMMENTS

1a Separate balloon distension resulted in a 20 % inhibition of submaximal pentagastrin-stimulated gastric acid secretion in 6 healthy subjects and produced no inhibition at all in 6 duodenal ulcer patients (I) Inhibition was a constant phenomenon in 30 healthy subjects (II) with an increase of the pH of the gastric contents during the distension period The mean output volume significantly decreased during the distension period with an almost complete recovery of phenol red (mean 92 96 %) throughout the experiments This excludes the possibility that the decrease in acid response was due to an increased alkaline reflux or an increase of alkaline secretion (40) in the stomach

The result suggests that antral distension elicits stimulatory a well as inhibitory mechanisms affecting gastric acid secretion in man The inhibitory mechanism seems to be defective in duodenal ulcer patients The inhibitory mechanism appears to predominate the balance in healthy subjects and thus the stimulatory mechanism is only detectable in duodenal ulcer patients In patients with an active duodenal ulcer the acid response to separ te antral distension amounted to about 35 % of the peak acid output after pentagastrin stimulation (12)

1b Neutralisation f the intragastri milieu with a pH increase from a mean of 2 7 to 6 8 during the distension period did not change the inhibitory effect f antral distension on pentagastrin-stimulated gastric acid secretion in six healthy subjects (II) Vagal stimulation produces a release of somatostatin in an acid intr gastric milieu in the cat (122) and in the pig (53) Somatostatin inhibits gastric acid secretion and gastrin release in the dog (5) and in man (97 100) In the dog an increase of the plasma concentration of gastrin has been observed during antral distension with an alkaline intragastric milieu but no increase occurred with an acid intragastr c milieu (29) In man no increase of the plasma concentration of gastrin has been observed during antral distension (11) irrespective of the pH of the intragastric milieu (10)

In the present study no change in the plasma concentration of

portal venous blood was observed in duodenal ulcer patients nor in non-ulcer patients (II) This strengthens the opinion that antral distension in man does not stimulate gastrin release In the present investigation (II) no change in the somatostatin concentration of portal venous blood was observed in non-ulcer patients nor was there any release of somatostatin into the gastric lumen in healthy subjects Unchanged plasma somatostatin and gastrin concentrations during antral distension indicate that this inhibition is not mediated via somatostatin (II) The inhibition of gastric acid secretion evoked by antral *distension which persisted during perfusion of the sto*mach with alkaline buffer also indicate that this inhibition is not mediated by somatostatin (II)

2 Antral distension resulted in a 20 % inhibition of gastric acid secretion stimulated by a continuous intravenous infusion of either betazole (Histalog) 1 0 mg kg b w ^{1} h or pentagastrin (IV) The plasma concentration of neurotensin in portal blood did not change in non-ulcer patients nor in duodenal ulcer patients during antral distension (IV)

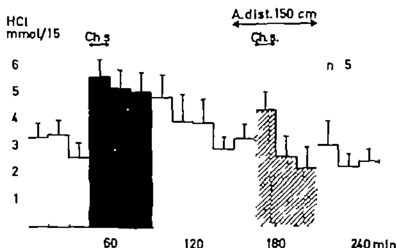
The gastrointestinal hormones secretin cholecystokinin and bulbogastrone are known to inhibit pentagastrin- but not histamine stimulated gastric acid secretion (69 68) Thus the consistent inhibition of Histalog-stimulated gastric acid secretion by antral distension indicates that it cannot be mediated by these gastrointestinal peptide hormones (IV)

The intravenous infusion of synthetic neurotensin inhibited pentagastrin-stimulated gastric ac d secretion in dogs (2) to approximately 50 % Therefore the concentration of immunoreactive neurotensin was determined during antral distension (IV) The plasma concentration of neurotensin remained unchanged and thus neurotensin could be excluded as a possible mediator of gastric acid inhibition produced by antral distension

Vasoacti e intestinal polypeptide (VIP) is known to inhibit both pentagastrin- and betazole stimulated gastric acid secretion (83 84) VIP could hypothetically be the mediator released by antral distension (36 25) Preliminary unpublished data has

shown a slight increase in the plasma concentration of VIP in portal blood during antral distension without any difference between duodenal ulcer patients and non-ulcer patients

a)



b)

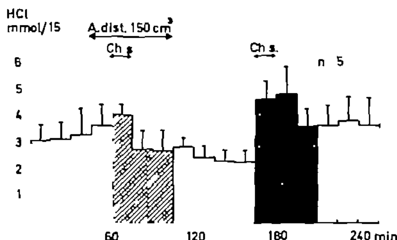


Fig 8 The effect of antral distension on gastric acid secretion stimulated by modified sham feeding Mean of 5 healthy subjects The hatched areas have been compared

a) The control test precedes the experiment

b) The experiment precedes the control test

3a The rapid onset of the inhibition of gastric acid secretion produced by antral distension and the immediate reversal of this inhibition mimicks the pattern of a reflex mechanism Moreover some further support to the inhibitory reflex theory is delivered by the 30 % 42 % inhibition of vagally stimulated gastric acid secretion in 5 healthy subjects (Fig 3a and b) whereas humorally stimulated gastric acid secretion in healthy subjects was inhibited to 20 % by antral distension (I IV)

Adrenergic reflex inhibition is an additional conceivable explanation Earlier investigations in dogs described a hyperchlorhydria after bilateral splanchnicectomy (89 92) and an increase in the occurrence of peptic ulcer after coeliac ganglionectomy (79) Sympathectomy in rats increased gastric acid secretion and the incidence of experimental peptic ulceration (90) Adrenergic transmittor antagonists stimulated gastric acid secretion (65 74) Beta adrenergic agonists have produced a marked inhibitory effect on gastric acid secretion (119 28 45) In a majority of the investigations it has not been possible to differentiate a direct adrenergic inhibition from an inhibitory effect secondary to diminished mucosal blood flow Some beta adrenergic agonists however inhibit gastric acid secretion without influence on the blood flow (28) In the present study neither $beta_2$ nor $beta_{1+2}$ adrenergic receptor blockade (III) changed the inhibition of pentagastrin-stimulated gastric acid secretion evoked by antral distension This suggests that the inhibitory effect of antral distension on gastric acid secretion is not mediated ia a beta-adrenergic reflex However the results do not exclude the possibility of an adrenergic inhibitory reflex mechanism Adrenergic inhibition on a ganglionic level by activation of alpha adrenergic receptors (125 133) may still be conceivable

3b The inhibition of basal and pentagastrin-stimulated gastric acid secretion induced by dopamine was abolished by an i m injection of 10 mg of metoclopramide in healthy subjects (24) This indicates a dopaminergic inhibitory effect which can be blocked by metoclopramide However a metoclopramide dose of 10 20 or 40 mg given intravenously had no effect on basal acid secretion or on maximal histamine-stimulated gastric acid

secretion in healthy subjects or duodenal ulcer patients before and after truncal vagotomy (88 87)

In the present investigation (IV) pretreatment with 10 mg of metoclopramide did not influence the inhibition of pentagastrin-stimulated gastric acid secretion induced by antral distension in 7 healthy subjects The results do not support the view that a dopaminergic mechanism acts as mediator for the inhibitory effect evoked by antral distension (IV)

4 Distension of the innervated stomach has been shown to stimulate pancreatic exocrine secretion (32 82 131 129 132 54 15) This response has proved to be dependent upon both vagi and splanchnics (130 132) The pancreas receives a mixed autonomic innervation involving at least cholinergi and adrenergic components (121 56) to both the exocrine and endocrine part of the pancreas Pancreatic glucagon is released by both cholinergic and adrenergic nerve stimulation (34) Insulin is released by cholinergic stimulation (98) and pancreatic polypeptide vi predominently vagal stimulation (109 114 108) A gastropancreatic reflex to the endocrine part of pancreas may exist

Separ te fundic and antral distension in man licited an increase in the secretion of pancreatic polypeptide (107) The cells secreting pancreati polypeptide are present both in the pancreas and in the gut (76) The endocrine part of pancreas is responsible for the release of pancreatic glucagon In the present study (V) the concentration of pancreatic glucagon in portal venous blood increased during antral distension

Proximal gastric vagotomy abolished the release of pancreatic polypeptide evoked by separate fundic distension (107) This supports the existence of a vagally mediated gastro-pancreatic reflex to the endocrine part of pancreas

In the present study a marked increase in the plasma pancre tic polypeptide concentration occurred in portal and peripheral venous blood following antral distension both in duodenal ulcer patients and in patients undergoing cholecystectomy (V) The concentr tion of pancreatic glucagon in portal venous blood

increased during antral distension in 15 patients (8 cholelithiasis patients and seven duodenal ulcer patients) (V) Neither the release of pancreatic polypeptide nor pancreatic glucagon can explain the inhibition of gastric acid secretion

The increase in concentration of pancreatic polypeptide in peripheral venous blood during antral distension was slightly suppressed by a $beta_{1+2}$-adrenergic antagonist A similar suppression did not occur after medication with $beta_1$ adrenergic antagonist (III) This indicates the possible involvement of a $beta_2$ adrenergic component in the release of pancreatic polypeptide

Separate antral distension in healthy subjects evoked a significant increase in the plasma concentration of motilin both with and without a background infusion of pentagastrin (unpublished observations) Motilin secreting cells are located predominantly in the jejunal part of the small intestine (19) This indicates that antral distension also influences the endocrine part of the jejunum by a possible gastrojejunal reflex?

Distension or mechanical stimulation of different parts of the gastrointestinal tract activates complex and differentiated mechanisms that are briefly outlined in Table I It appears that the functional involvement of the extrinsic and intrinsic control of the gastrointestinal tract in general differs considerably in its various compartments Nevertheless great similarities still exist and this is particularly so as regards the gastric and colorectal section Thus antral distension like distension or mechanical stimulation of the rectum elicits a complex response pattern activating both smooth muscles and the secretory glands Beside short or long nervous reflex activation a direct or indirect hormonal release appears to play an important part in these response patterns It appears likely that these reactions to distension (on meals or at defecation) are physiological and constitute well-graded reflex adjustments f the respecti rgan functions

Tabl I

SOME EFFECTS BY DISTENSION OF DIFFERENT PARTS OF GASTROINTESTINAL TRACT

DISTENSION OF	STIMULATORY EFFECT	RELAXATORY/INHIBITORY EFFECT	RELEASE OF GASTRO-INTESTINAL PEPTIDES
Distal oesophagus		Vago-vagal non-adrener gi recepti relaxation (86 66 1)	VIP (36)
Gastric fundus	Gastric acid secretion (51)		Gastrin in dog (30)
	Exocrine pancreatic secretion probably partly adrenergic (130)		Vagal pancreatic poly peptide (107)
Gastric antrum	Gastric acid secretion in duodenal ulcer patients (12)	Inhibition f gastric acid secretion in healthy subjects (1)	Gastrin in dog (29)
			Pancreati polypeptide release probabl partly $beta_2$-adrener gi (III)
			Pancreatic glucagon (V)
			VIP Motilin (unpublished)
		Vago-vagal non-adrenergic reflex relaxation (86 66 1)	
Small intestine	Contraction oral to stimulus (7)	Relaxation aboral to stimulus (7)	Serotonin (23 43)
			VIP (36)
		Intestino-intestinal inhibition (73)	
		Vasodilatation (13)	
Colon	Contraction oral to stimulus (8)	Relaxation aboral to stimulus (8)	Serotonin (77)
			VIP (36)
		Vasodilatation (intrinsic) (38)	
		Colo-colonic inhibition (73)	
		Vasodilatation (pelvo-pelvic) (38)	
Rectum	Pelvo-pel i coloni contraction and secretion (39)	Vasodilatation (39)	
		Anal relaxation (46)	

Different patterns of gastric acid secretion in duodenal ulcer patients and healthy subjects

A considerable number of differences in gastric secretory pattern have been established between duodenal ulcer patients and healthy subjects The cause of these differences is unknown as is the importance of these differences for the pathogenesis of duodenal ulcer disease (104) It has been suggested that certain patients illustrate a partial abnormality of gastric secretion (104) Duodenal ulcer patients have been found to have a significantly higher maximal acid response to pentagastrin (70) histamine (4 42) Histalog (134) and meal stimulation (42) However in one study (85) no difference was found in the peak rate of acid secretion in response to meal stimulation between duodenal ulcer patients and healthy subjects but the secretory response was prolonged in duodenal ulcer patients The increased capacity to secrete acid has been related to the greater number of parietal cells in duodenal ulcer patients (27) the cause of which is completely unknown

Duodenal ulcer patients display a greater increase in gastrin concentrations fter meal stimulation (126) or an intravenous infusion of isoprenalin (20) or adrenalin (21) than healthy subjects It has been suggested that there exists a G-cell hyperplasia in duodenal ulcer patients compared to healthy subjects (27) However the amount of antral and duodenal gastrin determined by biological methods (35) did not differ between duodenal ulcer patients and non-ulcer subjects

Another difference is the increased sensitivity for pentagastrin stimulation that has been observed in duodenal ulcer patients (63 96 103 116) A higher sensitivity for pentagastrin in duodenal ulcer patients might be in keeping ith an increased vagal tone (33) Indirect evidence speaks against vagal hyperactivity however a nce the frequency of high basal plasma concentrations of pancre tic polypeptide is the same in duodenal ulcer patients as n healthy subjects (109) Pancreatic polypeptide i mainly released by agal activation

Another conceivabl explanation of the increased sensitivity

of duodenal ulcer patients to hormonal stimulation are defective inhibitory mechanisms Several defective inhibitory mecha nisms have been established Acidification of the gastric contents has been found to reduce the inhibition of acid secretion stimulated by a meal (127) or by insulin hypoglucaemia (112) in duodenal ulcer patients compared to healthy subjects However defective acid inhibition of the gastric secretory response to a meal does not seem to be a constant finding in duodenal ulcer patients (117) Fat in the duodenum inhibited meal stimulated gastric acid secretion to an equal extent in duodenal ulce patient as in healthy subjects Howev r gastrin release was suppressed only in healthy subject (41) Submaximal pentagastrin-stimulated gastrin acid secretion was significantly less inhibited in duodenal ulcer patients compared t healthy subjects when small volumes (20 ml) or oleic acid were infused into the duodenum (71)

The present study has established another abnormality in the acid secretory patt rn f duodenal ulcer patients i e the defective inhib tion of acid secretion by antral disten ion in duodenal ulcer patients (I) Antral distension off rs certain advantages in lucidating abnormalities of the secretory pattern of duodenal ulcer patients The experimental model is imple and the inhibitory ffect i absent in duodenal ulcer patients but is constantly observed in heatlhy subjects Gastrointestinal peptides with properties t inh b t gastri acid secretion do not seem to medi te the inhibitory ffect of antral distension The data obtained complies with a non-bet adrenergic non-dopaminergic reflex inhibitory mechanism possibly acting on a ganglioni level

SUMMARY

Balloon distension of the antrum in healthy subjects decreased basal acid secretion and consi tently resulted in a 20 % inhibi tion of pentagastrin-stimul ted gastric acid secretion independent f the ntraga tric pH Antral di tens on stimulated gast ic acid secretion in duodenal ulcer patients and did not evoke any change f pentagastrin-stimulated acid secretion The plasma concentration of gastrin in portal and peripheral venous blood

did not change in duodenal ulcer patients nor in non-ulcer patients during antral distension Thus antral distension evoked both an inhibitory and a stimulatory effect on gastric acid secretion in man The inhibitory mechanism predominated in healthy subjects and was defective in duodenal ulcer patients The stimulatory mechanism in duodenal ulcer patients is probably a reflex activation and gastrin release from the antrum does not participate

The nature of the inhibitory mechanism was investigated in healthy subjects Antral distension inhibited pentagastrin- and Histalog-stimulated gastric acid secretion to the same extent This excludes gastrointestinal peptides such as secretin cholecystokinin and bulbogastrone as possible mediators of the inhibition of gastric acid secretion

During antral distension the concentrations of somatostatin and neurotensin were unchanged in portal and peripheral venous blood Somatostatin was undetectable in the gastric contents Antral distension released pancreatic glucagon and pancreatic polypeptide into portal venous blood in amounts which are probably insufficient to inhibit gastric acid secretion Pancreatic glucagon pancreatic polypeptide and vasoactive intestinal polypeptide (VIP) were released in similar amounts by both duodenal ulcer and non-ulcer patients The results indicate that no gastrointestinal peptide with an established acid inhibitory property mediated the inhibitory effect of antral distension on gastric ac d secretion i healhty subjects The results support however the concept of gastro-pancreatic reflex to the endocrine part of the pancreas with release of at least pancreatic polypeptide and pancreatic glucagon

The rapid onset of the inhibition of gastric acid secretion produced by antral d stension and the immediate reversal of this inhibition mimicks the pattern of a reflex mechanism The inhibitory effect of antral distension remained however fter $beta_1$ and $beta_{1+2}$ receptor blockade and fter an intramuscul r inject on of the dopaminergic antagonist metoclopramide An inhibitory reflex acting at the ganglionic level is supported to some extent by preliminary data showing that antral distension

inhibited vagally stimulated gastric acid secretion more efficiently than pentagastrin-stimulated gastric acid secretion in healthy subjects

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RELATION BETWEEN

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RELATION BETWEEN MECHANICAL AND MORPHOLOGICAL CHARACTERISTICS IN URINARY BLADDER SMOOTH MUSCLE

BY
BENGT UVELIUS

LUND 1980

This summary contains apart from previously unpublished data results from the following papers

I UVELIUS B 1976 Isometric and isotonic length-tension relations and variations in cell length in longitudinal smooth muscle from rabbit urinary bladder Acta Physiol Scand 97: 1-12

II UVELIUS B 1977 Influence of muscle length on the force-velocity relation of K -contractures in smooth muscle from rabbit urinary bladder Acta Physiol Scand 101: 270 277

III JOHANSSON B HELLSTRAND P & UVELIUS B 1978 Responses of smooth muscle to quick load change studied at high time resolution Blood Vessels 15: 65-82

IV UVELIUS B 1979 hortening velocity active force and homogeneity of contraction during electrically evoked t itches in smooth muscle fron rabbit urinary bladder Acta Physiol Scand 106 481 486

V UVELIUS B & Hellstrand P 1980 Effects of phasic and tonic activation on contraction dynamics in smooth muscle Acta Physiol Scand in press

VI UVELIUS B & GABELLA G 1980 Relation between cell length and force production in urinary bladder smooth muscle Submitted to Acta Physiol Scand

The papers are referred to in the text by their Roman numerals

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CONTENTS

1 GENERAL INTRODUCTION

Due to intense research over many years our present knowledge is large regarding morphology energetics mechanical properties etc of skeletal muscle The sliding filament concept of contraction was established some 25 years ago (for review see e g Huxley 1971) Research on smooth muscle mechanics has been hampered for several reasons The experimental procedures have been difficult to standardize and it has been difficult to obtain reproducible results Katz showed however already in 1939 that Hill s (1939) equation is applicable also to smooth muscle by experiments on tortoise retractor penis muscle In more recent years Katz results have been confirmed and extended for a variety of mammalian smooth muscles (e g Åberg & Axelsson 1965 Hardung & Laszt 1966 Stephens Kroeger & Mehta 1969 Mashima & Handa 1969 Gordon & Siegman 1971 Hellstrand & Johansson 1975) From these studies it can be seen that smooth muscle in general has lower maximal shortening velocity (V_{max}) than skeletal muscle and that isometric tension for most preparations is of the same magnitude as for skeletal muscle There is great variation between results obtained from different smooth muscles regarding V_{max} Whereas V_{max} was only 0 03 lengths/s for rabbit taenia coli at 22°C (Mashima & Handa 1969) it was 0 74 l/s for rat portal vein at 37°C (Hellstrand & Johansson 1975) Part of this difference is probably due to different methods (afterloaded isotonic contractions or quick release) and temperature but most probably different smooth muscles possess indi idual cha acteristics

The mechanical behaviou of a smooth muscle depends (among other thin s) on the characteristics of the contractile protei s and on the relation between neighbouring cells since smooth muscle prepa ations are multicellula During recent years it has been shown (see Somlyo & Somlyo 1975) that smooth muscle contains thick and thin myofilaments although not as regularly arranged as in skeletal muscle The intercell lar relations are complicated In t enia coli from guinea pig (Gabella 1976c) the cells are anchored to each other and to collagen fibrils along the cell surface and at the end of the cells complicated interdigitations are observed

If mechanic l properties determined fo smooth muscle preparations are to be onsidered rele ant for the individual cell one has to know how the c lls behave i compa ison to the whole preparation e g how the lengths of the cells change in proportion to a length pertubation of the whole

preparation The most straight-forward way to avoid this difficulty is to study the behaviour of isolated individual cells It is possible to obtain viable single cells from toad stomach digested with trypsin and collagenase (see review by Fay Cooke A Canaday 1976) Such cells can be mounted for recording of contractile force during activation for instance by electrical stimulation It is not certain however that the mechanical output of an isolated cell represents the behaviour of a cell in situ anchored as it is not only at its ends but also laterally to neighbouring cells and to collagen fibrils present in the space between the cells

Another way to obtain information regarding the mechanics of the individual cells is to perform the relevant experiments on multicellular preparations in which the behaviour of the individual cells in relation to the preparation at a whole is known With this approach and the above mentioned background the present study was performed

Scope of present investigation

The preparations used are rabbit and guinea pig urinary bladder Some experiments are performed on whole bladders in vivo and others on strips of longitudinal bladder muscle in vitro The reason for the choice of this preparation (which has not been used much previously for mechanical studies) was mainly that the length of the isolated preparations could be increased considerably without any marked increase in passive tension The bladder as a densely innervated organ with only minor spontaneous activity is also easy to control and stimulate Some comparison is made with results on rat portal vein which has considerable passive tension and a phasic spontaneous activity This is done with the notion that conclusions regarding smooth muscle in general can be drawn with greater certainty if comparative results are obtained from different smooth muscle types

The results of the morphological and morphometrical studies of the bladder prepa ations are accounted for in chapter 2 The effects of bladder distension on cell length and cell diameter in whole bladders and of length changes n isolated strips are shown

Chapte 3 deals ith the mecha ics of bladder muscle Length-tension relations for single smooth muscle cells re calculated from volume-acti e -pressu el t ons in vivo An analysis is given of the applicability of the isoton c quick release method (Jewell & Wilkie 1960) fo the study of

mechanics of isolated strips The behaviour of the muscle strips during active shortening and the effect on this of different modes of stimulation are investigated A study of the capability of the bladders to adapt to an introduced residual volume is given in chapter 4 Chapter 5 contains a general discussion and a summary

2 MORPHOLOGICAL ASPECTS

2 1 Introduction

The mammalian smooth muscle cell in general is thin and slender The dimensions vary depending on the organ and on the functional state (se rev by Burnstock 1970) The mid-cell diameter ranges from 1 5 to 6 µm and the length from 30 to 450 µm Earlier ultrastructural studies of urinary bladder smooth muscle cells (Nagasawa & Suzuki 1967 Larsen 1977) show that bladder cells conform to this general description The length and diameter of rabbit bladder cells were estimated to be about 200 µm and 5 µm for relaxed and 100 µm and 9 µm for contracted cells (Larsen 1977) Both thick and thin myofilaments were observed Available data is however not sufficient for a proper understanding of cellular events in relation to the behaviour of the bladder as a whole The aim of the studies reviewed in this chapter is to provide such quantitative information that will be necessary for the interpretation of the results from the mechanical experiments in chapter 3 From a description of the remodelling of the muscle wall when the bladder is inflated (2 3 2:4) attention will be focused on the behaviour of the single muscle cell in situ Cell length (2:) and number of cells per mm^2 (2:6) will be described in relation to bladder radius In 2 7 an attempt will be made to determine how cell volume is affected by bladder distension Finally (2 8) morphological characteristics are given for the isolated bladder strips used in chapter 3

2 2 Outline of methods

Adult guinea-pigs (body weight 300-500 g) and rabbits (body weight 2500-3500 g) were used The general processing is outlined below There will be div rgences from this scheme in section 2:8 but they will be accounted for there

The animal was killed and the bladder was excised emptied and transferred to oxygenated Krebs solution (paper VI) The urethra was then cannulated and the bladder slowly filled to a desired volume either through a gravity fall or by stepwise injections with a syringe connected to the cannula Filling c uld take p to 30 min fo the highest volumes The bladder was then transfe red to glutarald hyde solution at room temperature After about 10 min the bladder was cut open di ided into identifiable parts hich were transf rred to fresh fixative Some bladders were fixed with their content of urine immediately after ligation of the rethra and removal

from the animal After 10 min i fixative the bladder was slit open and the content volume was measured The bladders were then treated as described in the foregoing The preservation of fine structures was not good in these bladders perhaps due to leakage of urine into the bladder wall during fixation

After the fixation period (2-6 h) the specimens were immersed for 1-2 h in 2 % osmium tetroxide and then transferred for 30 min to a 1 % aqueous solution of uranyl acetate After dehydration with ethanol and expoxypropane the specimens were infiltrated with Araldite After polymerization suitable blocks were cut with glass knives Sections 1 5 - 3 5 µm thick for light microscopy were collected on microscope slides ex mined and photographed unstained in a phase contrast microscope Sections for electron microscopy were collected on grids stained with lead citrate and examined in a Philips 300 electron microscope All histological observations were carried out on detrusor muscle from the mid-ventral part of the bladder

When cross sections should be made on muscle bundles in a block the exact direction of the bundle was visualized under a dissection microscope and appropriate cuts could be made Bladders with small content volumes were not translucent enough to make it possible to see the bundles Under such circumstances it was necessary to make serial sections for phase contrast microscopy to ensure that a bundle was at right angle to the direction of the cuts Generally a bundle was followed over at least 1 mm

Bladder radius ill be e pressed as bladder oute radius This is alculated from the bladder outer volume hich is obtained by the addition of the bladder all volume to the conte t volume The reason fo doing this is that the muscle layer is much clos r to the outer than to th inne s rface due to a submucosa of consid ble thickness I other experiments the weights of gui ea pig bladders ere found to be a ound 0 3 g and therefore 0 3 ml i dded to all content volumes i order to obtain outer volum s Me n rabbit bl dd r weight was 1 8 g and 1 8 l is added to th content volumes in rabbit bladders in order to obtain outer volumes

Bladde radius and circumfe ence re c lc lated from bladde volume assuming sphe ical form The gu nea-pig bladder is in fact almost spherical at all volumes studied (0 6 7 ml) h e s the abbit bladde is slightly elong ted

Quantitative results are given as mean ± standard error (SE) Number of observations are given in parenthesis

2 3 Variation in the muscle layer thickness with changes in bladder volume
If distension of the bladder portion that is used in this study (the midventral portion) is to be calculated from the bladder volume the assumption has to be made that all parts of the bladder wall are stretched by the same relative amount in response to increases in bladder volume It can be expected a priori that one part of the bladder wall namely the trigonum is more resistant to stretch than the rest but the trigonum constitutes only a small portion of the bladder wall In this section the applicability of the assumption that all parts of the guinea pig bladder are stretched to the same extent will be considered

Araldite-embedded midventral portions from 6 guinea pig bladders were used The bladders were fixed with content volumes between 0 25 ml and 6 7 ml The muscle wall thickness was measured with a calibrated eye piece in the phase-contrast microscope 15 determinations were performed for each of the six bladders The results are shown in Fig 1 It is seen that the *muscle wall thickness decreases when the bladder outer* radius is increased At a bladder outer radius of 0 51 cm (corresponding to a content volume of 0 25 ml and an outer volume of 0 55 ml)

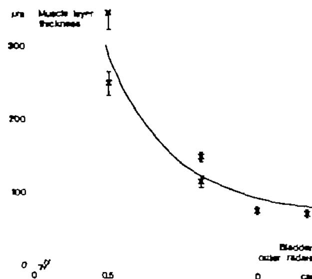


Fig 1 Thickness of total muscle layer of 6 guinea-pig bladders filled to different volumes Bladder outer radius represents radius of the content assuming spherical shape plus total bladder wall thickness Full line indicates expected behaviour calculated from the volume of the muscle layer at the lowest bladder volume assuming that all parts of the bladder are equally distensible Each point represents mean of 15 thickness det rminations

the mean muscle layer thickness of the 2 bladders is 300 µm This gives a calculated total volume of the muscle layer of 92 mm^3 If all parts of the bladder wall are stretched to the same extent when the bladder is inflated and if the volume of the muscle layer can be considered independent of content volume muscle layer thickness should vary in accordance with the full line in Fig 1 The thickness measurements agree well with what is to be expected if the above mentioned assumptions are releva t

The results in this section thus show that a certain relative increase in guinea pig bladder radius results in the same relative length change in the particular segment of the bladder wall used in the e periments (the mid--ventral portion)

2 4 Rearrangement of muscle bundles with increasing bladder volume
The muscle wall of the bladder is considered to consist of three ill-defined layers an outer and an inner longitudinal and an intermedi te circular layer (see e g Ham 1969) The circular layer is usually the thickest This description seems to be valid also for the bladders used in this study Each layer seems to consist of muscle bundles seperated from each other by connective tissue The direction of the bundles in each layer is not uniform Bundles with a direction almost at right angle to the others are often found in the different layers The expressions circular and longitudinal layer therefore express only th general direction of the muscle bundles within each layer

Th thickness of the muscle lay r as a whole decreases when the bladder is inflated as shown in section 2 3 In the phase-contrast microscope it can be seen th t cross-sectioned bundles in sections from distended bladders seem to be somewhat flattened when compared to bundles from bladders ith a low content volume This flattening is however not prominent The profiles of individual cells in cross-sectioned bundles are by judging from the electron micrographs somewhat flattened in the distended bladders but much less so than would be expected if the bundles ere stretched later lly in proportion to the increase i bladder diamete

Th phase-contrast microg aphs (VI) suggest that the muscle bundles spread laterally hen the bladder is inflated i e that the radial numbe of bundles decreases with increa ing bladder volume This as a general

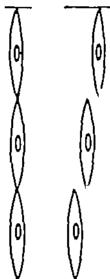
impression for the 6 guinea-pig and 8 rabbit bladders studied If this is correct the number of muscle cells across the thickness of the wall would decrease when the bladder is inflated Therefore the number of muscle cells across the full thickness of the muscle coat was determined by counting how many muscle cells were intersected by radial lines drawn orthogonally through the wall These determinations were performed either on phase-contrast micrographs or directly in the microscope The results show that the radial number of cells decreases with increasing bladder radius for all volumes studied (VI) This is to be expected if as suggested above the muscle bundles spread laterally when the bladder is inflated

There are two additional conclusions to be drawn from the results presented above 1 it is difficult if not impossible to dissect out strips for in vitro experiments where all cells have the same direction Even in strips from one muscle layer bundles with a considerable oblique direction in relation to the long axis of the preparation will be included This will be of relevance for the in vitro determination of force per unit cross sectional area (see chapter 3) 2 as the muscle bundles in the wall tend to spread tangentially more than being flattered when the bladder is inflated length variations for isolated strips (here the bundles are not flattened) will be a good model of the individual bundle in situ If the bundles had become considerably flattened in situ the geometrical arrangements of the cells within a bundle might have changed with bladder volume Then it would have been much more difficult to transfer results from strips i vitro to the hole bladder

2 5 Muscle cell length in relation to bladder distensio

If results from mechanical experiments on whole bladders or bladder strips are to be carried over to indi idual cells in the preparation it is essen tial to know how the cells are coupled to each other Fig 2 shows schemati cally 2 extreme ar angements i e series (SC) and parallel coupling (PC) I termediates between these are also possible If the individual cells in the figure produce the same force then the force output of the model ith 3 par llel coupled cells would be 3 times greater whereas the SC p epa ation g es just the same force as one cell If the length of the prepa ations is doubled by passive st etch then the cell length in the SC model is also doubled whe eas cell length for the PC model increases to a much greate e tent These are drastic examples showing th necessity

Fig 2 Alternative arrangements of muscle cells within a tissue The cells coupled in parallel (right) produce per unit cross sectional area 3 times the force attained by the preparation with the cells coupled in series (left) The non-cellular force-transmitting structures in the right panel are assumed to be rigid Modified from Murphy (1976)



of knowing the cellular coupling if cellular behaviour in intact preparations is to be calculated

If cell lengths are determined for preparations subjected to different degrees of stretch the cell la coupling can be determined (see Murphy Driska & Cohen 1977) Should the cells be coupled in series a direct proportionality between changes in preparation length and cell length is to be expected Should they be parallel coupled the relative length change of the cells is greater than for the preparation as a whole the magnitude being dependent on the degree of parallel coupling

In orde to study the dimensions of individual cells Anopolsky (1928) obtained suspensions of isolated smooth muscle cells from pig fallop an tube by maceration of unfixed tissue pieces in 30 % nit ic acid Cooke & Fay (1972) macerated gluterald hyde fixed strips of guinea-pig taenia coli in 25 % KOH Th method used in pape I is a modification of the method devised by Anopolsky Rabbit bladd rs we e filled to desired content volumes and placed for 1 h in Ca-fre Krebs solution containing 1 0 mM EGTA in order to chelate Ca^{2} and thereby ensure that the cells ere in a r la ed state The bladde s were fi ed in an isotonic buffered formaldehyde fixative After 24 h the fixative longitudinal strips of the bladders

were dissected out These strips were then placed in increasing concentrations of nitric acid 1/2 h in each starting with 0 6 % (roughly isosmolar) The strips were finally placed in 30 % HNO_3 for 4 days The rationale for the stepwise increase in concentration was to avoid possible effects on the cells such as shrinkage induced by drastic changes in osmolality of the bathing medium When the macerated strips were gently stirred after 4 days in nitric acid a suspension of isolated cells and cell conglomerates was obtained A drop of this suspension was placed on a slide and a cover slide was put on Isolated individual cells were photographed and the lengths were measured on the prints Only isolated intact symmetrically formed cells with well tapered ends were considered

In order to ascertain that the muscle cells were not activated during fixation and that they did not shrink when they were placed in the nitric acid solutions tension of some longitudinal strips was measured continuously during these procedures No tension developed which strongly suggests that the cells were fixed in a relaxed state

Filled circles in Fig 3A show the results of the cell length determinations It is clearly seen that an increase in bladder radius is accompanied by a closely similar relative length change of the individual cells The results agree qualitatively with those of Cooke & Fay (1972) on guinea-pig taenia coli although they have studied cells from only two preparation lengths The results are also well in accordance with those on pig carotid artery reported by Murphy Driska & Cohen (1977) Driska & Murphy (1978) and Driska Damon & Murphy (1978)

There is some uncertainty regarding the reliability of the above study since the length of the cells might possibly change during the maceration of the strips despite that no tension de elopment was observed Such tension measurements ca probably not account for all possible changes in cell length during the maceration espec ally not during the later phase when the cells begin to loosen from each other It was also felt that length changes if they occured might be of diffe ent importance at different degrees of stretch

The most reliable data regarding cell length are most probably obtained from ser al ele tron microscope sampling such studies have been done (e g Bennet & Rogers 1967 Merrillees 1968) but the approach has not been

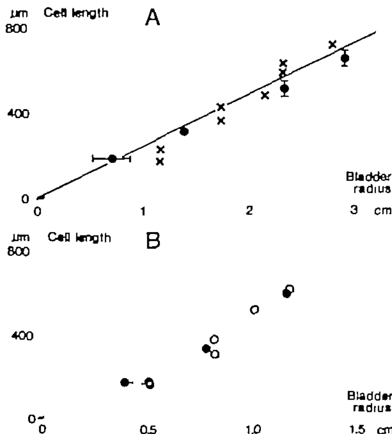


Fig 3 Muscle cell length vs bladder outer radius A Rabbit Filled circles indicate determinations on nit ic acid macerated bladders 3 bladders at each volume Vertical bars indicate SE (n 53 79) horizontal bars wall thickness Crosses show morphometrically determined lengths of longitudinal muscle cells The line shows the e pected behaviour if the cells were coupled in series B Guinea-pig Open circles longit dinal muscle filled circles: circular muscle For longitudinal muscle radius is gi en as outer radius for circular muscle as inne r d us Poi ts connected by broken lines indicate results from same bladde From (I) and (VI)

applied to the study of different deg ees of stretch It would probably be too laborious to obt in a reasonable number of length d terminations in such an investigation

Halpern Mulvany & Warshaw (1978) hav used Noma ski inte ference contrast optics to measure distance bet een intracell lar structu es of the same muscle cell in segments of small at mesente ic arte ies in itro Increases

in internal circumference by up to 50 % in inactive vessels resulted in a nearly proportional increase in spacing between the intracellular markers The results regarding the part of the muscle cell between the markers thus correspond well with those described above These authors have however no results regarding the behaviour of the tapering ends of the cells

Gabella (1976a) has devised a morphometric method to determine muscle cell length

$$\text{mean cell length} = \frac{\text{mean nucleus length} \quad 100}{\text{\% of cross-sectioned cell profiles containing nuclei}}$$

The advantage of this method is that the cells are left in situ thus minimizing the risk that length changes could occur This method was applied to 6 guinea-pig and 8 rabbit bladders Cross sections for EM were cut from identified longitudinal muscle bundles and the number of cross sectioned cells were counted as were the number of cells containing nuclear profiles Then the tissue blocks were re-embedded in Araldite and tangential sections for phase-contrast microscopy were cut from the bundles chosen above Lengths of nuclei with identifiable nuclear poles were measured with a calibrated eye piece With guinea pig bladder the same method was used also for determining cell length in the circular muscle layer The number of cross sectioned cell profiles counted in each determination ranged bet een 448 and 2252 and the number of nuclei between 26 and 115 The majority of the cell profiles are smooth indicating that these cells are fixed in a relaxed state A fraction of the cells have however rough surfaces and protrusions which indicates that these fibres were fixed in an activated state (regarding such protrusions see e g Gabella 1976b)

The calculated cell length determinations are shown for rabbit bladder cells in Fig 3A and they are similar to those obtained by nitric acid maceration It thus appears that the less tideous nitric acid method gives data as reliable as those obtained by the method of Gabella The full line in Fig 3A shows the expected cell length variations in relation to rabbit bladder radi s f the cells were coupled in series The experim tal data fit closely to this line According to Fig 3B a certain relative inc ease in guinea pig bladde d us is accompan ed by a slightly large relative length increase f th muscle cells This suggests that there may be some degree of pa allel oupl ng in th s preparation The de iation from the

series coupling behaviour is however so small that the cells in the guinea pig bladder can be considered in general to be coupled in series

It is not known if smooth muscle cells shorten when activated if the preparation as a whole is kept at constant length The bladders used for nitric acid maceration were pretreated with EGTA and no comparison as made with bladders fixed while activated The morphometric determinations described above were done on bladders that were not depleted with Ca^2 the fixative itself had 0 5 mM Ca^2 added The electron-micrographs also showed that some cells were fixed in an activated condition Despite this cell length was almost the same as for the macerated inactive bladders The cell length determinations by Driska & Murphy (1978) on pig carotid artery *were done on K activated strips alone Mulvany and Warshaw (1979) were* unable to measure distance between intracellular markers for activated mesenteric arteries in vitro due to movements within the preparation They found however that nucleus length as on average little changed This suggested according to these authors that cell length was on average not substantially affected by activation Fig 4 shows nucleus length vs bladder radius for rabbit bladder It is seen that bladder radius and thus cell length can vary considerably without much change in length of the nucleus At least for this preparation nucleus length does not seem to correlate ell with cell length

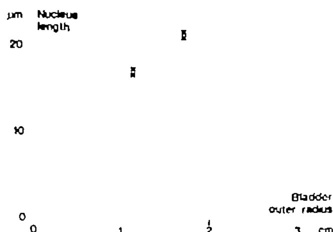


Fig 4 Length of nuclei in longitudinal muscle from rabbit bladd r Each point repr sents mean value from 35 nuclei i one bladde 8 bladders we e used Only nuclei with all identified clear zones around thei nucl a pol s were consid red

While no conclusive results exist regarding lengths of activated cells vs passive it my impression that such length changes if they occur are small This statement based on the fact that no significant length difference was found between passive macerated bladder muscle cells and morphometrically determined cell lengths of bladders fixed in the presence of Ca^2 where some cells showed signs of being fixed while active (Fig 3A)

2:6 Number of cells/unit area Dependence on bladder volume

It was shown in section 2:5 that cell length increased in a bladder muscle preparation when the length of the preparation is increased The number of cells per unit area (cell packing density) in cross-sectioned muscle bundles would then be expected to rise with increasing stretch of the preparation

Results regarding cell packing density of guinea pig taenia coli strips fixed at different lengths have been reported (Gabella 1976b) In one experiment a taenia strip was fixed at resting length whereas a contiguous strip was allowed to shorten actively down to 24 % of resting length Packing densities were 94000 cells mm^{-2} and 18000 cells mm^{-2} respectively In a similar experiment the packing density was 105000 mm^{-2} in the stretched strip and 37000 mm^{2} in the strip fixed when shortened to 40 % of initial length

In the present study on guinea pig urinary bladder cross-sectioned muscle bundles were marked out on EM-plates (VI) the cell numbers were counted and the contours of the bundles were copied onto transparent film sheets These contours were then cut out and weighed By knowing the weight of a calibration area the cross sectional area of the bundles could be calculated Fig 5 gives the results Each point is based on 184-405 cells For the whole radius interval studied cell packing density increases when the bladder is inflated The straight line obtained by linear regression analysis fits well (r 0 91) with the experimental data

Only four rabbit bladders were studied In the two preparations fixed with a content volume of 5 ml packing density in the longitudinal muscle was 86000 and 64000 per mm^2 respectively From the mean values of these (75000 mm^{2}) the figure at 20 ml would be expected to be 119000 mm^{2} ($4^{1/3}$ 75000) The actual data for two bladders fixed at this volume were 117000 and 141000 (mean 129000) which is close to the expected value

Number of cells/mm²

Filled symbols: circular cells

Open symbols: longitudinal cells

y 24504 150022X

r 0.912

150000

100000

50000

0

0 0.5 1.0 cm

Bladder radius

Fig 5 Number of cells per mm^2 cross-sectional area vs bladder outer radius Broken lines connect values from same bladder The best fitting line according to linear regression analysis is given From VI

The results in this section as ell as those in section 2 5 (increase in cell length with bladder radius) suggest that no slippage between cells occur even at great distension of the bladder wall The importance of this finding will be discussed in chapter 3 with regard to possible explanations for the broad and flat length-active tension curve that will be shown to exist for this type of muscle

2 7 Effects of passive stretch on ell volume

According to Fay & Delise (1973) smooth muscle cells from toad stomach decrease in volume when they shorten actively As the cells shortened to 30 % of their esting length the calculated cell volume decreased to 80 % of the initial value

The results from paper VI repo ted under 2:5 and 2 6 permit us to speculate on the effect of bladde distension on cell volume Fig 6 is a composite of the data in Figs 3B and 5 The relation between cell length and cell pa king density is well approximated by a straight line in that a doubling of cell length is accompanied by a doubling of the cell packing density This would indicate that cell volume does not change with length if the

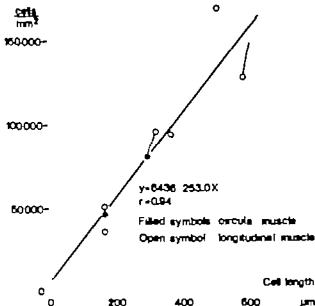


Fig 6 Number of cells per mm² cross-sectional area vs cell length The figure is composed of results from Fig 3B and Fig 5 Broken lines connect results from same bladder Best fitting line obtained by linear regression analysis is shown

amount of intercellular space was constant The problem of the constancy of this space with changes in muscle length has not yet been investigated by use of specific tracers such as Co EDTA However microscope observations by Gabella (personal communication 1980) indicated that the size of the extracellular space is length-independent in guinea-pig taenia coli

While the results of my own studies may not be conclusive on this point they do suggest that cell volume does not change much when cell length is aried The discrepancy between these findings and the results of Fay & Delise (1973) might be due to the fact that they studied active shortening while the changes in bladder cell length were passi e

2 8 General morphological description of isolated strips used for mecha ical e periments

The strips that ere used for mechanical experiments in chapter 3 were blotted and weighed afterwards in order to determine their cross sectional area This made them unsuitable for histological study For the latter purpose strips composed mainly of longitudinal muscle were dissected out f om rabbit bladders filled with 10 ml Krebs solutions Either the bladders ere fi ed before dissection or the strips w re fixed at their in situ length

In one series of 6 strips the preparations were fixed in glutaraldehyde embedded in plastic and cross-sectioned for phase-contrast microscopy From photomicrographs the relative area of smooth muscle was measured to be 67 ± 3 %

In another series of 6 strips the tissues were fixed in Bouin s solution and then paraffin-embedded Cross sections and longitudinal sections 7 µm thick were cut and stained with resorcin-fuchsin and picric acid - thiazin red for differentiation between smooth muscle elastic fibrils and collagen (Romeis 1968) The muscle bundles were found to be surrounded by loose connective tissue containing considerable amount of collagen The relative cross-sectional area consisting of muscle was the same as for the series described above Only a very limited number of elastic fibrils was seen among the muscle bundles A high amount of elastic fibrils was found around intramural vessels and in concomitantly embedded rat portal veins

On electron-micrographs of both rabbit and guinea pig bladder collagen fibrils are seen surrounding the individual muscle cells No f rther study regarding their relationship to the muscle cells was done (regarding cell-collagen relationship see Gabella 1977)

3 MECHANICAL PROPERTIES

3 1 Introduction

Active force produced by a skeletal muscle increases with increasing extension of the muscle until a maximum is reached Further stretch leads to a progressively decreasing active force The mechanism behind this length-active tension relation was long obscure but certain features of it has now been adequately explained (Gordon et al 1966) The decrease in force seen when the muscle is stretched beyond the optimal length coincides with the decreasing overlap between the thick and thin myofilaments Thus fewer cross bridges can participate in force production The decrease in force at lengths shorter than optimal has been more difficult to explain An increasing internal load could be of importance in this part of the length tension diagram (Gordon et al 1966) but evidence has been presented that the electromechanical coupling especially in the central parts of the muscle cell becomes less efficient at shorter lengths (Rüdel & Taylor 1971)

Smooth muscle has a length-tension relation that in principle has the same features as that of skeletal muscle (see e g Speden 1960 Csepo 1962 Stephens Kroeger & Mehta 1969 Halpern Mulvany & Warshaw 1978 paper I) An important difference is however that smooth muscle preparations can develop tension at much shorter length relative to the optimal than skeletal muscle Section 3 3 will deal with the length-tension relation of rabbit bladder strips and possible explanations regarding the shape of it Section 3 4 contains an attempt to determine the length active tension relation of single guinea pig bladder cells

The relationship bet een load and shortening velocity is an important cha acte istic of the contractile machinery Hill (1938) in a classical *se es of e periments sho ed that sho tening velocity (V) can be described* by a hyperbolic function of the force (P)

$$V(P \quad a) \quad b(P_o \quad P) \qquad \text{eqn } 1$$

This equation represents a rectangular hyperbola with asymptotes at $V = \underline{-b}$ and $P \quad \underline{a}$ and a P axis intercept at $P \quad P_o$ the isometric force Until recently t as thought that the constant a reflected directly the e ergy liberated dur ng shortening Later investigations ha e cast doubts on this direct relation bet een thermal and mecha ical events (see e g Simmons &

Jewell 1974) The force-velocity relation is however still of great importance as a means of characterizing properties of the contractile system

The muscle model suggested by Hill (1938) separates a contractile component and an undamped series elastic element During an isometric contraction the contractile element was thought to shorten thereby stretching the series elastic element A sudden change in the load of an isotonically shortening muscle was considered to cause an immediate adjustment of the length of the series elastic element and of the shortening velocity of the contractile component according to its force-velocity relation Thus with this model no transient behaviour is to be expected Experiments with this theoretical background has been performed on smooth muscle which has been found to behave qualitatively as other muscle types V_{max} is however much lower than in skeletal muscle (see e g Stephens Kroeger & Mehta 1969 Gordon & Siegman 1971 Herlihy & Murphy 1974 Hellstrand & Johansson 1975)

During recent years the two-component model by Hill has been challenged mainly as a result of new studies on skeletal muscle A majo part of the elasticity of the acti e muscle in well controlled studies is now considered not to be directly in series with the contractile machinery but to be associated with the cross-bridges themselves (Huxley & Simmons 1971 Ford Huxley & Simmons 1977) Moreover the shortening velocity does not change instantaneously to a new steady level after a load change The adjustment of the kinetics of the crossbridges takes a certain time (Civan & Podolsky 1966) The same is the cas with regard to force following a length step (Hu ley & Simmons 1971 Ford Huxley & Simmons 1977) Velocity and force transients have recently been observed also in smooth muscle (Study III; Hellstrand & Johansson 1979; Mulvany 1979)

The mechanical characteristics of strips of longitudinal smooth muscle from rabbit u inary bladder at different lengths (3 6) and with diffe ent modes of activation (3 7 3 8) have been studied using the isotonic quick release method devised by Jewell & Wilkie (1960) for skeletal muscle The series elastic element the force-velocity elation and the velocity transients will be cha acterized (3 5 3 7) An analysis of the applica bility of the quick release method for the present purpose will be gi en in the methods chapter

3 2 Outline of methods

The following refers to the experiments described in sections 3 3 3:5 3:6 3 7 and 3 8 The methods used in 3 4 will be described in that section

Rabbits weighing between 2 and 3 kg were killed by a blow on the neck The bladders were dissected out and transferred to oxygenated Krebs solution Longitudinal strips containing principally longitudinal muscle (see 2 8) were dissected out from bladders filled with 10 ml Krebs solution The in-situ length of the preparation at 10 ml will be referred to as L_{10} This was generally about 5 mm The preparation weight was from 0 5 to 4 mg The strip was then mounted in an apparatus that could keep the muscle isometric and when desired release the muscle to previously determined afterloads 3 types of apparatuses were used For the determinations of length-tension relations in section 3:3 the apparatus described by Hellstrand Johansson & Ringberg (1972) was used The isotonic lever had an equivalent mass about 2 5 g This made it unsuitable for the study of fast events but it was appropriate for the studies in 3 3 Part of the experiments in 3 3 and all *experiments of 3 6 and 3 8 were performed with in an improved apparatus* (regarding the design see: Johansson 1973) The equivalent mass of its lever was 150 mg and the compliance of the whole system was 10 µm/g The force and velocity output was recorded on a linear direct-writing oscillograph (Devices MX4) This apparatus gave adequate registrations from about 50 ms afte the release The period 0-50 ms was disturbed by oscillations originating mainly in the force transducer To enable registration of the events immediately after a release a further improved apparatus was used Its lever has an equi alent mass of 8 mg (for further details see Sjölin Hellstrand & Clementz 1978 Hellstrand & Johansson 1979) The output of the force transducer and of the photo electric device registering lever movement ere displayed on a sto age oscilloscope (Tectronics 530IN) and recorded by a G ass kymogr ph camera Simultaneous slow recordings on a linear direct iting oscillograph (Devices MX4) were used to monitor the experiments The signals we e also stored on magnetic tape (Tandberg 100 or Thermio ic Store 4 instrumentation recorder) for further analysis This setup was used for sections 3 5 and 3 7

The preparations were kept in a solution of the following composition in mM NaCl 120 KCl 6 0 $MgCl_2$ 1 2 $CaCl_2$ 5 glucose 11 5 and tris(hydroxymethyl) aminomethane (Trizma Base Sigma Chemical Co) 23 (It is referred to s N tris) The solution had previously been titrated with HCl at 37°C

to pH 7 4 The osmolality measured by means of an Osmometer 31 LAS (Advanced Instruments Inc) was close to 300 mOsm The solutions were continuously bubbled with 100 % O_2 The preparations were allowed to accommodate for 1 h before the experiments were started Two means of stimulation were used K^+-contractures were elicited by changing the N-tris solution to a solution (K-tris) in which all NaCl had been exchanged with equimolar amounts of KCl The muscles could also be stimulated electrically with square waves or AC current through platinum electrodes

Passive tension was 2-4 % of maximum active tension unless otherwise stated Results are given as mean ± SE

According to Edman & Nilsson (1972) the degree of damping of the lever is critical in experiments on the mechanics of heart muscle due to effects of oscillations on the contractile function If the lever was underdamped the inertial oscillations were found to deactivate the muscle and the shortening velocity was impaired Reservations against the damped-release method has however been put forward (Gülch 1974) The releases in the present studies are all undamped To find out whether no deactivating effects of inertial oscillations occur in bladder preparations experiments of the following design were made Bladder strips ere stimulated electrically Early during the rising phase of one contraction the muscle was allowed to perform an fterloaded shortening Later du ing another contraction the muscle was eleased to the previous afterload If no inactivation occurred during the quick r lease the shortening velocity at a given degree of shortening should be the same for the aft rloaded contraction (devoid of oscillations) and after the quick rele se (possessing oscillations) Fig 7 gi es the results of one experiment Th two shortening curves superimpose ell o cept during the first 75 ms after the quick rele se Du ing 0 - 75 ms shortening elocity is actually higher after the quick r lease The obvious conclusion must be that inertial oscillations after quick eleases have no deactivating effects on the smooth muscle preparation

3 3 Length tension relations of bladder st ips in vitro
Strips containing longitudinal smooth muscle from rabbit urinary bladder we e used (I) After the accommodation period the strips rela ed in Ca free N-tris and were then transf red for 5 min to K tris solution which licited a cont acture tension reaching a plateau level in 1 2 min The bathing solution was then changed back to Ca^2 free N tris and the strips

Fig 7 An afterloaded isotonic contraction and a quick release to the same afterload of an electrically stimulated rabbit bladder strip (length 6 0 mm) In order to superimpose the length registrations the contraction ending with an afterloaded isotonic shortening is delayed in the figure relative to the quick release response The length change in the quick release registration is typical: an initial elastic recoil followed by an intermediate phase of rapid shortening and finally a slower shortening that can be superimposed upon the afterloaded shortening The intermediate phase last about 75 ms

rapidly relaxed Time in this solution was 10 min These steps were then repeated at different muscle lengths The experiments generally started at L_{10} and the muscles were then shortened in steps When enough data had been obtained the muscles were stretched in steps After the experiments the strips were gently blotted and weighed Their cross sectional area at the length where they developed maximum active tension was calculated assuming a density of 1 0 mg/mm^3 Fig 8 shows the length-tension relations for 5 strips treated as just described Active tension (total minus passive tension) starts to develop at a length of about 35 % of L_{10} Active tension increases with stretch until a maximum is reached at a length of 106 ± 4 %

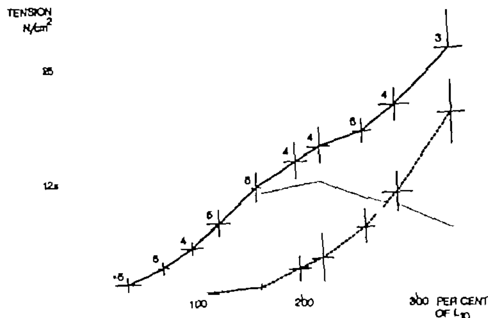


Fig 8 Mean length-tension relations of 5 strips of longitudinal smooth muscle from rabbit urinary bladder L_{10} is the length of the strip at a bladder volume of 10 ml Relative lengths are divided into 10 classes Continuous line Total tension Broken line Passive tension Dotted line Active tension The bars indicate SD From I

of L_{10} (n 5) Maximum active tension was 12 5 ± 0 4 N/cm² Further stretch results in decreasing active and increasing passive tension After correction for non-muscular tissue (see 2 8) mean active tension is 19 N/cm²

The shape of the length-tension curve obtained here is typical for mammalian smooth muscle (see e g Stephens Kroeger & Mehta 1969 Lowy & Mulvany 1973) The bladder strips begin to develop tension at a length about 17 % of the optimal This agrees well ith the 13 25 % obtained by Stephens et al (1969) and differs ma kedly from skeletal muscle which do not normally develop tension at lengths shorte than 65 % of optimal (Gordon Huxley & Julian 1966) The absence of Z-discs in mammalian smooth muscle might be of importance for their ability to shorten extensively It could also be possible that the lesser efficiency of the electromechanical coupling at short muscle lengths reported by Rüdel & Taylor (1971) does not apply to smooth mus le but there is no evidence for this As shown in chapter 2 no slippage between the muscle cells xist for a bladder radius interval cove ing the whole rising part of the length-tension curve Slippage can

therefore be ruled out as a reason for the slow rise in active tension relative to increase in muscle length

Increasing the Ca^{2+} concentration in the K tris solution to 10 mM doubled the contractile force (I) but the shortest length at which active tension occured was virtually unchanged Shortening down to 17 % of optimal length thus probably represents the maximal isotonic response Lowering of extra cellular Ca^{2+} to 0 5 mM diminishes isometric force by a factor of ten compared to force output in 10 mM Ca^{2} The shortening capacity decreases however only slightly

It has been found for skeletal muscle that the active tension developed at a certain length is the same irrespective of whether this length is reached passively or by isotonic shortening from a greater length (Gordon et al 1966) It was observed in the present studies (I) that this is not always the case for bladder strips A series of 6 experiments was performed to compare isometric and isotonic length-tension relations The strips were activated by K-tris following the same time schedule as described above and isometric tension as measured At every second contraction the muscle was allowed to perform afterloaded isotonic shortenings and its length was varied as above Up to initial lengths about 165 % of L_{10} the tension produced at a certain length was the same irrespective of how that length was reached At lengths above 165 % of L_{10} the muscle could no longer shorten isotonically to the point on the isometric length-tension curve corresponding to the afterload This shortening defect which was most pronounced for intermediate and high afterloads increased progressively ith f rther increases in length At very small loads the shortening defect as almost absent To see if the shortening defect could be due to e g overstretch of injured tissue i the knot region three experiments with the following design were performed Three 0 1 x 0 5 mm silver markers were placed bet een the muscle bundles in the preparation thereby dividing it into four segments Isometric and isotonic contractions were performed and the dist nces between the markers ere measured w th a dissection microscope The inability of the muscles to shorten isotonically fron large starting lengths to corresponding points on the isometric length-tension curve was apparent and th s sho tening defect occurred in all four segments i e also in the cent al ones hich were unaffected by knot injuries

Differences between isometric and isotonic length tension curves resembling those presented above have been noted also by workers using other smooth muscles e g Dobrin (1973) on dog carotid artery and Stephens & Van Niekerk (1976) on dog trachealis muscle The phenomenon in these tissues seems to be at least partially reversible and differs in this respect from the irreversible changes described by Peterson & Paul (1974)

No satisfactory explanation for the shortening defect is available It could be possible that cell slippage exists at extreme lengths but according to section 2 5 the relationship between bladder radius and cell length is linear up to 100 ml volume which corresponds to 215 % of L_{10} and the shortening defect appears already at initial lengths from 165 % of L_{10} As the tension plateau during a K contracture lasts for a considerable time the possibility of a decrease in activation with time as an explanation is unlikely

The shortening defect is probably of minor physiological impo tance as it appears only at lengths that correspond to bladder volumes near or above the normal maximum Ruptures of the mucosa often occur in the rabbit bladder at 100 ml content volume (corresponding to 215 % of L_{10})

3 4 Calculation of c ll lar length-active tension relations

The smallest functional component in skeletal muscle is the sarcomere In smooth muscle it is not known whether a corresponding structural unit exists or not At present th ll has to be considered the smallest functional part of smooth muscles Isomet i twit h s have been elicited in isolated single cells f om toad stomach (Fay t al 1976) and shortening velocity was recorded i such prepar tions by Bagby (1974) To my knowledg no length tension rel tions of single isolated cells have been reported

Where a singl isol ted cell produ for only end-to-end it is possible that cells in sit ca transmit force o er many pa ts of their s rface to neighbouring lls I thi s ction ill try to calc late length- ct e tension relations f a mean guinea p g bladder ell in s tu from olume-active p essur relations obtained in ivo The experiments we c rried out on ether-anaesthetized gu nea-pigs weighing about 400 g Th abdomen w s opened and the bladde was annulated i the u ethra The et rs we e ligated and cut 0 5 1 cm f om the bladd and e e d awn tog ther with surrounding ti sue through pl stic tubes w th platinum electrodes at each

end The electrodes were connected to Grass S44 stimulators via a Grass SIU5 isolation unit The cannula was connected to a Statham P23 DC pressure transducer Through a branch tube the bladder could be filled with Krebs solution to desired volumes starting at zero After the pressure had stabilized the bladder was stimulated for 15 s by a train of supramaximal square waves Pressure increased rapidly to a maximum and then declined to some extent before the stimulation was ended The bladder was filled to a new volume and the procedure repeated When the highest desired volume was reached the bladder was emptied in steps and stimulated again Time between stimulations was 10 min Fig 9A summarizes the results Maximum active pressure was obtained at 0 15 ml bladder volume and pressure in the relaxed bladder was negligable at all volumes Assuming a spherical form which is

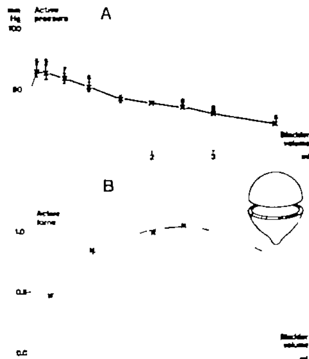
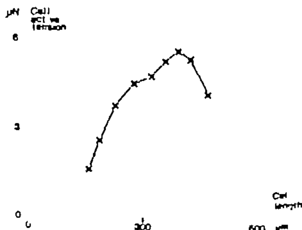


Fig.9 In vivo experiments with bilateral pelvic nerve stimulation Guinea-pig A: Active pressu e vs content volume Four animals were used Mean ± SE Numbers of determinations indicated in the figure Maximum active pressure occurs at 0 15 ml B Force produced by the longitudinal muscle holding the two indicated hemispheres together vs bladder volume Maximum force at 2 5 ml From VI

an appropriate approximation (see above) the volume–pressure relation can be transformed to a volume-force curve The force trying to separate the two hemispheres in Fig 9B is $F = r^2 \pi P$ (r is bladder radius P is pressure) This separating force is balanced by the force produced by the longitudinal smooth muscle F A T (A area of longitudinal muscle T is tension /unit of area/ The active force developed by the wall of the bladder reached a maximum of 1 04 N at 2 5 ml bladder volume (Fig 9B)

The total number of longitudinal muscle cells transsected according to the bladder model in Fig 9B was calculated Mean thickness of the longitudinal layer was measured on the sections used in 2 3 The thickness times the circumference gave the area of longitudinal muscle at the equatorial region From Fig 5 the relevant cell packing densities could be obtained The total number of cross-sectioned longitudinal cells at the equatorial region was 185000 ± 24000 (n 6) Using this figure the forc /cell could be calculated at all volumes in Fig 9 From Fig 3B the corresponding cell lengths could be obtained Fig 10 shows the results of these calculations in the form of a cellular length-tension curve A maximum active force of 5 5 µN is developed at a cell length of 400 µm This maximal force per cell is comparable to those obtained by Fay (1977) in isolated toad stomach cells and by Mulvany & Halpern (1976) in vascular smooth muscle If the length-active tension curve is extrapolated downwards by a straight line the length corresponding to zero tension becomes 110 µm which is 28 % of the optimal length This is higher than the 17 % obtained in 3:3 for whole

Fig 10 Calcul ted length active tension relation for a average individual longitudinal smooth muscle cell of guinea-pig urinary bladd r From VI



preparations of rabbit bladder strips but this difference may just reflect uncertainties in the calculations

The number of cells per mm^2 at optimum length is 107000 according to Fig 5 Active force per cm^2 pure muscle bundle would then be 59 N This value is high compared to most other vertebrate smooth muscles including those used for the results in section 3:3 One reason is probably that our in vivo experiments are performed on bladders with good metabolic conditions (no impairment of blood flow) and in contrast to strip preparations there are no injuries caused by knots or dissection procedures Gabella (1976c) showed that guinea pig taenia coli strips produced more tension with carbachol than with K high solutions or electrical field stimulation He obtained 42 N cm^{-2} with carbachol corresponding to 74 N cm^{-2} when correction was made for nonmuscular tissue Stimulation of the pelvic nerves to the bladders could on these grounds be expected to give a more powerful contraction than what is obtained by K tris in 3 3

The reason for the high active force in some mammalian smooth muscle is not known Rosenbluth (1965) showed that the myofilaments of toad jejunum cells are not in parallel with the long axis of the cells This could be a factor that increases cellular force However according to Gabella (1979c) myofilaments in taenia coli cells appear almost parallel to the long axis of the cells at all degrees of shortening Myosin filaments are 2 µm long in rabbit portal vein (Ashton Somlyo & Somlyo 1975) compared to 1 6 µm for skeletal muscle (Page & Huxley 1963) According to Rüegg (1971) active force in muscle is directly proportional to thick filament or sarcomere length Longer thick filaments enable more cross-bridges to work in parallel thereby increasing force output Maximum active force for mammalian smooth muscle is about 30 N/cm^2 (Close 197) If everything else was const t 41 N/cm^2 (30 2 2/1 6 N/cm^2) would be expected in skeletal muscle if the thick filaments had the same length as in smooth muscle The v riatio i th ck filament length can thus not account for the whole diff rence The significan e of the high actin and low myosin content in smooth compared to skeletal muscle (Murphy et al 1974) remains to be assessed Smooth muscle has lower tension cost than skeletal muscle (Paul et al 1976 Hellst nd 1977 Johansson 1978) suggesting that the duration of the cross-bridg cycle is longer If this was due to a longer relative time at the att ched state than in skeletal muscle this would give a high fo c output

3 5 Isotonic behaviour after quick release

In Fig 7 a typical quick release is shown The behaviour after the release can be divided into 3 parts There is an immediate recoil followed by a phase of fast shortening lasting 50 – 75 ms Finally the shortening slows down and superimposes almost exactly on a corresponding afterloaded isotonic shortening curve This section will deal with the three phases of the quick release response in the order that they appear

The immediate recoil has usually been attributed to the series elastic element according to Hill s (1938) two-component model (see e g Jewell & Wilkie 1958 Edman & Nilsson 1972 Hellstrand & Johansson 1975) In smooth muscle the recoil of this series elastic (SE) element when the muscle is released to zero tension varies considerably The earlier the study the more compliant is the SE element found to be [Åberg (1967) 13 % guinea-pig taenia coli; Stephens & Kromer (1971) 7 5 % dog trachealis muscle; Meiss (1978) 4 % rabbit mesotubarium] This may partly be due to different preparations and methods in the different studies Fig 11 shows SE

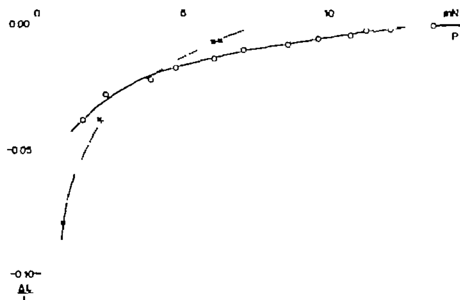


Fig 11 Series elastic recoil of a rabbit bladd r str p and a rat po tal vein Open circles: Bladder (length 4 76 mm isomet ic forc 3,0 N/cm²) Crosses Po tal vein (length 5 00 mm isometric force 1 7 N/cm²) Fits to eqn 2 are shown by full and broken lines respectively Constant k is 62 for bladder and 36 fo portal vei indicating that the series elastic element is more complia t in the latte AC stimul tion

load extension-curves in a rat portal vein and a rabbit bladder preparation Despite the fact that the same apparatus and the same mode of stimulation were used there is a considerable difference in SE recoil between the two muscles The experimental data could be fitted to an exponential equation of the following form

$$P \quad A \exp(k \times \Delta L/L) + B \qquad \text{(eqn 2)}$$

The stiffness $(\frac{\Delta P}{\Delta L})$ increases in proportion to force with the proportionality constant k In paper III k was 91 ± 13 (n = 7) for urinary bladder and 28 ± 5 (n = 5) for portal vein In paper V k was 53 ± 6 and 34 ± 4 respectively The difference in urinary bladder stiffness between the two studies may be related to differences in preparation length and active tension development It is not known which structures that are responsible for the SE recoil In skeletal muscle mounted under strictly controlled conditions the recoil is considered to be due to elastic behaviour of the cross-bridges themselves (e g Huxley & Simmons 1971) The SE recoil in the bladder muscle is however several times larger than in skeletal muscle (regarding the latter see e g Bressler & Clinch 1974 Ford Huxley & Simmons 1977) It is therefore unlikely that a major part of the series elasticity would reside in cross bridges in bladder muscle but it has been suggested that a small fraction may reside in these structures (Hellstrand & Johansson 1979) Meiss (1978) calculated the extension of the SE element for one isolated toad stomach muscle cell in the study by Fay (1977) and obtained a value 2 2 % of the cell length This is only somewhat smaller than the 3 4 % maximal SE recoil in bladder muscle found in study III This comparison could indicate that also in bladder muscle a major portion of the series elastic element resides in structures on or in the cell At present no clearcut explanation for the difference bet een bladder and portal ein exists A difference in cell size could play a role but the cell packing density in rat portal vein (66000 mm ^{2} Uvelius unpublished) is not very d fferent from what is found in bladder (Fig 5) Another possibility is that extracellular structures are of importance One differenc observed between the two muscle types in the present studies is the richer amount of elastic fibrils in portal vein This could explain not only the high passive tension in portal vein but perhaps also part of the high ompl ance of its SE element

The intermediate phase of the quick release response characterized by fast shortening was first investigated in (III) It has since been described and analyzed by Hellstrand & Johansson (1979) and Hellstrand (1979) It seems possible that it is an effect of cross-bridge rearrangement and change of kinetics after the release This transient phase subsides after about 75 ms after the release (III)

Determinations of shortening velocity for force-velocity curves were performed at 100 ms after release thus avoiding effects of the transient As shown in Fig 12 the experimentally determined force-velocity points can be fitted to the Hill equation The curve-fitting was performed either by using a linearized form of Hill s equation (II) or by a computer program that took into account errors in both coordinates (III IV V) As will be seen in the following sections V_{max} varies with muscle length and mode of stimulation

3 6 Influence of muscl length on force-velocity relations
In this section K^+ activated rabbit bladder strips were used (see II) Vmax as 0 29 ± 0 03 l/s (n 8) at L_{10} When the muscle was stretched P_o (the isometric tension) in reased according to the length-active tension r lation of the muscle (see 3 3) V_{max} was also found to be length-dependent even when it was expressed in relation to actual muscle length At L_{10} a/P_o was 0 17 ± 0 02 (n = 8) and b was 0 052 ± 0 008 muscle lengths per second (n 8) Thes values are of the same order of magnitude as those found for instanc by Mashima & Handa (1969) Stephens et al (1969) and Herlihy & Murphy (1974) As a and b no longer have any certain relation to th real events in muscle there is no point in discussing the small differences that exist Within the interval 0 69 L_{10} - 1 44 L_{10} b was constant hereas P_o/a varied in relation to P_o indicating th t lso a was length independent ov r this interval Th results co respond well ith those of Gordon & Siegman (1971) who also found a and b to be constant although the length interv l was smaller in their study

Hill s equation can be rewritten as V [b/(P a)](P_o - P) With const t alues for b and a and with P (the afte load) kept unchanged in the e p ment V should y in proportion to (P_o - P) Releases to small aft loads were performed i a separate study The results indicated that there is a line r lationship between P_o and V at lengths up to about 1 25 L_{10} Th s means that up to 1 25 L_{10} the length-active fo ce relation and the

length - V_{max} curve vary concomitantly with muscle length At lengths greater than this shortening velocity increases more than (P_0 - P) The reason for this is not clear There might be a length-effect on the para meters a and b at these degrees of stretch It is also possible that the force carried by the contractile units is less than P due to an unloading effect of the passive parallel elasticity which begins to carry force at these lengths

3 7 Effects of different modes of activation on contraction dynamics

In sections 3 5 and 3 6 it was shown that Hill s equation fits well with observed shortening velocities against different loads When P_0 was altered by a change in muscle length V_{max} was affected to almost the same proportion This could lead to the assumption that there exists a unique relationship between P_0 and V_{max} It has been suggested however that V_{max} in smooth muscle may vary with the mode of stimulation being higher during activation by AC current than during K^+-contractures (Hardung & Las t 1966 Hellstrand & Johansson 1975 Murphy 1976) On the other hand P_0 seems also to be higher with AC stimulation (Gabella 1976c) It would be of great interest if it could be shown conclusively that V_{max} is affected differently than P_0 with different modes of stimulation (AC current K tris) since this finding would indicate that the kinetics of the contrac tile process is different in tonic (K^+) and phasic (AC) activity The problem has not been studied systematically so far

In (V) 2-3 min long K^+-contractures are elic ted in rabbit bladder strips At the peak of each contracture a quick release was made using different afterloads After a sufficient number of such responses had been recorded the muscle as kept in N-tris and repeatedly stimulated by AC current (25—100 Hz 2 - 4 V duration 2 5 s) at 1/2 - 1 min interval Suprama imal AC stimulation usually ga e higher isometric tension than obtained in the K contractures (*cf Gabella 1976c*) *Therefore the intensity of the AC* stimulation s adjusted to g ve the same P_0 as the preceding K contrac t res

The init al (SE) re oils at different afterloads were fitted by a computer program to eqn 2 (s above) The cha act ristics of the elastic element were found to be un ffected by mode of stimulations the constant k of eqn 2 was 51 ± 6 (n 6) f AC stimul tions and 54 ± 3 (6) for K^+ contractures Hellstrand & Johansson (1979) found that the series elast city was

unaffected by changes in temperature of the bathing medium This together with the apparent lack of influence of stimulation mode (as contrasted to the marked effects on V_{max} see below) suggests that a major part of the SE recoil is a passive phenomenon

The length response after the elastic recoil was fitted by a computer program to a double-exponential model (Hellstrand & Johansson 1979) of the form

$$L(t) = L + A_1[1 \quad \exp(-t/\tau_1)] \quad A_2[1 - \exp(t/\tau)] \qquad \text{eqn } 3$$

The amplitude (A_1) of the fast shortening component was unaffected by mode of stimulation (paper V) The rate constant ($1/\tau_1$) was however about 25 % smaller in K^+-contractures A comparable effect is seen when bladder muscles are cooled (Hellstrand & Johansson 1979) Their suggestion that the fast shortening component is not a passive phenomenon is further validated by the present results The fast exponential component subsides apidly and is practically over at 100 ms after the releas the time at which shortenin velocity was measured The force-velocity values were fitted by a computer program to eqn 1 in 3 1 V_{max} was found to be about 30 % lower in K - contractures than in responses to AC stimulation [0 26 0 03 (n 6) l/s vs 0 37 ± 0 05 l/s] Force-velocity elations from a typical muscle is shown in Fig 12

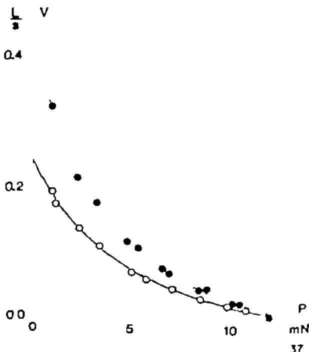


Fig. 12 Force-velocity relations of a bladder strip Velociti s measured 100 ms after the release Filled circles AC-stimulation open circles K -activation Fits to Hill s eqn are shown by broken and full lines respec tively From V

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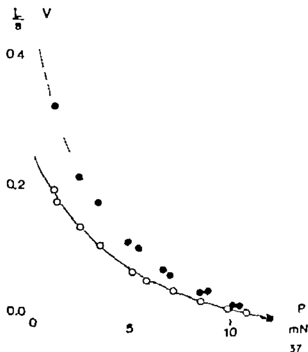


Fig. 12 For e-velocity relations of a bladder strip V locities meas red 100 ms after the release Filled circles AC stimulation open circles K^+ activation Fits to Hill s eqn re shown by broken and f ll lines r spec tively From V

There is no straight forward explanation for the fact that V_{max} is lower in K^+-contractures than in AC-contractions of equal P_o It has been reported by Jones et al (1973) that vascular smooth muscle cells swell and that their thick myofilaments disrupt when the vessels are placed in KCl high media These authors suggested however that the disruption of the thick filament lattice took place during the fixation of the tissue for electron microscopy They base this suggestion on the fact that the tension output was the same as for K_2SO_4 solutions which did not cause swelling of the cells The difference in shortening velocity described above could be an effect of filament disruption if this change occurs also in the living muscle

Another factor which could contribute to the different V_{max} values is the time the muscle is activated before the releases are made The AC contractions are short-lasting and the releases are performed after 2 5 s whereas 1-2 min elapse before a K^+-contracture reaches its maximum K^+ activated slow fibres from Xenopus laevis shorten more slowly if a release is made late during a contraction compared with an early release (Lännergren 1978) The finding in paper IV that V_{max} declines faster than P_o during a single twitch might also be of relevance Peiper (1979) found that the rate of tension recovery in portal vein after vibration-induced relaxation is slower late during a K contracture Altogether these results might indicate that the rate of the bridge-cycling is slowed down with time during prolonged contractions The findings of Arner & Hellstr nd (1980) that in relation to tension output the oxygen consumption decreases with time during K^+-contractures further support this idea A possible explanation could be that an internal load develops during long-lasting contractions e g due to rigor comple es (Weber & Murray 1973) caused by insufficient ATP supply

The V_{max} value fo rabbit bladder muscle (0 37 l/s V) is high compared to many other smooth muscles but much lower than in skeletal muscle (from 7 l/s; Close 1972) The possible relationship between V_{max} and metabolic rat in smooth muscle is discussed by Hellstrand (1977)

3 8 Single t itch s Shortening locity and acti force

Paper IV gives an account on how P_o and V_{max} are affected in rabbit bladde st ips at 25° C du ing single t itches elicited by single supramaximal square wave pulses P_o and V_{ma} were determined as the intercepts with th

force and velocity axis respectively of the computer fits of the data to Hill s equation

The time from stimulation to peak of the twitch was about 2 s V_{max} and P_o reached their maximum values at the same time about 1 s after stimulation V_{max} then declined rapidly At the peak of the isometric force for instance the V_{max} value was only 70 % of its maximum The P_o value on the other hand decreased more slowly and is at the peak of the isometric contraction only slightly less than maximum A comparison of force-velocity curves from the rise and from the peak of the isometric response shows that they differ in the same manner as the curves obtained during AC and K stimulations respectively in 3 7 The possibility that inhomogeneity of activation could be of importance for the change in the force-velocity relation during the twitch was ruled out Strips ere marked with charcoal grains and were photographed while contracting Only minor movements of the grains were observed

The difference in time between the releases during the rise and those at peak of contraction is smaller than the difference bet een el ses performed during AC and K contr ctions They strengthen howe er the suggestion in 3 7 that the duration of activation is of importance for V_{max} rather than the mode of stimulation

In response to an increased functional demand smooth muscle will hypertrophy (see e g Gabella 1975) The development of the hypertrophy is fast as seen e g in rat portal vein subjected to increased intramural pressure (Johansson 1976) The cross sectional area of longitudinal muscle in this *vessel has increased considerably within 8-11 days after the pressure rise* This fast hypertrophy shows that there exists an intimate dynamic relation between muscle cell size and the functional demands

Peterson et al (1974) have induced hypertrophy in rat urinary bladder by injection of paraffine into the lumen In the present study this method was used on female guinea-pigs weighing 550 to 600 g Paraffine with a temperature just above melting point (43° C) was injected through a urethral cannula into the bladder of nembutal anaesthetized animals Each animal had a matched control with the same body weight Terramycin was added to the drinking water in order to avoid bladder infection Despite this precaution half of the paraffine-injected animals had to be discarded due to loss of weight probably as a result of bladder infection

After 10 days the animal was killed and the bladder was emptied of urine leaving only the paraffine mass A longitudinal bladder strip was marked out measured dissected free and mounted in an apparatus that could record isometric tension and isotonic shortening (lever equivalent weight 300 mg; see 3 2) The strip was stimulated by K tris solution following the same time schedule as in 3 3 The minimum length to which the muscle could shorten ctively when unloaded as determined as was the length at which maximum active tension d veloped Thes lengths will be expressed in relation to the in situ length of th strip After the experiments the strip was blotted and weighed The bladder was weighed befor and after 3 days in 70° C i order to determine the water content of the tissue The volume of th paraffine mass was measured The control bladder was filled ith Krebs sol tion to give a content volume equal to the paraffine olume A strip as then marked out and treated as described above

Table I gi es the results from the 4 hypertrophic bladders and from their controls The bladder eight incr ased from 0 43 0 03 g in the controls to 1 30 ± 0 17 g *The control bladders eighed slightly more than guinea-pig* bladders i chapte 2 (0 3 g) probably because the animals used here were

larger There is little inflammatory reaction in the bladders of these selected animals The increase in bladder weight is therefore not due to inflammatory oedema The water content did increase however from 78 ± 0 9 % in the controls to 86 ± 0 3 % in the hypertrophic bladder The study did not give any indication regarding structural localization of the increase in water content

There was no significant difference in optimum length for force generation but this length is difficult to determine due to the broad and flat length-tension curve The tension output for both controls and hypertrophic bladders in Table I was low compared to those in 3 3 and 3 4 The main reason is probably that the present strips contained all layers (mucosa to peritoneum) and no correction for the amount of non-muscular tissue has been made The active tension per unit area was considerably lower in hypertrophic bladder (0 91 ± 0 23 vs 1 67 ± 0 15 N/cm^2) The same qualitative difference has been described by Johansson (1975) for portal vein

Table I A comparison between hypertrophic (H) bladders and their controls (C) Content volumes between 1 2 and 2 1 ml The length(L_{max}) at which maximum active tension (P_{max}) is developed is expressed in relation to the in situ length of the strip Passive and active tension at L_{max} are expressed in relation to both wet eight/L_{max} and dry weight/L_{max} The length (L_0) that the unloaded muscle can shorten to is given in relation to the in situ length The results we e analyzed according to student s t-test for paired data p < 0 05 ** = p < 0 01 ** p < 0 001

	C	H
bladder et weight (g)	0 43 ± 0 03	1 30 ± 0 17
bladder dry weight (g)	0 09 ± 0 01	0 19 ± 0 02
water content (%)	78 ± 0 9	86 ± 0 3
$L_{max}/L_{in\ sit}$ (%)	136 ± 15	133 ± 10
$P_{passive}$ t L_{ma} wet (N/cm^2)	0 62 ± 0 19	0 42 ± 0 17
$P_{passive}$ at L_{ma} dry (N/cm^2)	2 89 ± 0 95	2 99 1 4
P_{max}; wet (N/ m^2)	1 67 0 15	0 91 ± 0 23
P_{max}; dry (N/cm^2)	7 74 0 85	6 6 ± 1 55
L_0/L_i situ (%)	60 ± 7	80 ± 8

and Gabella (1979b) for intestinal muscle P_{max} per cm^2 dry muscle would be 7 7 ± 0 9 N/cm^2 for the controls and 6 3 ± 1 6 N/cm^2 for hypertrophic bladders These values did not differ significantly and might indicate that the decreased tension per unit area seen in hypertrophic bladders is due to water accumulation Gabella (1979b) observed a considerable increase in intermediate filaments and a relative decrease in myofilaments and he suggested that this might be a factor contributing to the relative reduction in contractile force He also suggested that the changes in *intercellular collagen that he describes might be of importance In the* present study passive tension at L_{max} was higher for control bladders (0 62 ± 0 19 vs 0 42 ± 0 17 N/cm^2) but if passive tension is considered in relation to dry cross-sectional area the difference disappears This could indicate that the structures responsible for passive tension are not considerably changed in hypertrophic bladders

Unloaded hypertrophic strips can shorten less relative to their in situ lengths than their matched controls (Table I) This indicates that the relation between the muscle cells or the properties of the contractile proteins within the cells have changed According to Gabella (1979a) cell length is unchanged in intestinal muscle 3 5 weeks after onset of hypertrophy Shortening capacity was not determined in that report and the time before sacrifize was long r than in this study If in spite of this his results are applicable to the present study they might indicate that the properties of the myofilaments ha e altered Comparisons of characteristics of the force-velocity relations bet een controls and hypertrophic bladders would obviously be of great interest Such studies have not yet been performed It is to be noted however that V_{max} is lower in papillary muscles from hypertrophic hearts compared to controls (Hamrell & Alpert 1976)

5 GENERAL DISCUSSION

This discussion can be separated into 4 parts 1 The relation between preparation length (or bladder distension) and muscle cell length 2 the effect of preparation length on tension development (length-tension relations) 3 the isotonic behaviour of bladder muscle at different lengths and stimulation modes and 4 effects on length-tension relations induced by bladder hypertrophy

The cell lengths were measured in papers I and VI using two different methods The direct relationship seen between preparation length (or bladder radius) and cell length strongly suggests that the cells are coupled in series This is in accordance with the results from pig carotid artery by Murphy Driska & Cohen (1977) No slippage between the muscle cells could be detected even at considerabl stretch Evidently results regarding tension output and shortening velocity vs e g preparation length represent in a direct way the behaviour of the individual muscle cells

As there exists a linear relationship between cell length and number of cells per unit cross sectional area it seems reasonable to assume that cell volume is independent of the degree of passive stretch Fay & Delise (1973) found that the stereometrically det rmined volumes of isolated muscle cells from toad stomach decreased up to 30 % when the cells were stimulated and actively shortened The diff rence in results could indicate that her as passiv length changes do not induce net wat r movement over the cell membrane active shortening does

The length-tension relation of bladd r muscle has the same general configuration as that of other smooth muscles (for ref see Murphy 1976) Rabbit bladder st ips can sho ten to 17 % f the optimal length for acti e tension development The low i e i active tension hen a bladder strip is stretched is as mentioned above not due to cell slippag As the cells are coupled in s ries the same length-tension behaviour is to be e pected for individual cells The study includes an attempt to det rmine length- ti tension r lations for single cells in int ct guine -pig bladders (VI) Maximum acti e tension (5 5 µN per cell) is attained t a ell length of 400 µm This cti e force c rresponds to 59 N per cm^2 a high value compared t 19 N pe cm^2 obtained for rabbit bladde st ps It appe rs unlikely that this difference would be due to the diffe ent species sed It is probably so that the int ct guinea pig bladd r t

has a better supply of oxygen and nutrients and that there is not tissue damage of the kind expected to occur when strips are dissected out These favourable conditions do not apply to the isolated rabbit strips

The capacity of smooth muscle to shorten extensively in comparison to skeletal muscle could be due to the absence of Z-discs and/or to better electromechanical coupling at shorter lengths Variations in extracellular Ca^{2+} greatly influences force output in bladder muscle but the capacity to shorten is not much changed Active force per unit cross sectional area in guinea-pig bladder muscle is higher than in skeletal muscle Part of this might be explained by longer thick myofilaments as described in other smooth muscles(2 µm vs 1 6 Ashton et al 1975) The cell length determinations in the present study (I VI) show that parallel coupling which would increase tension output is virtually absent The lower tension cost observed for some smooth muscles (Paul et al 1976 Hellstrand 1977) might indicate that in these muscles the crossbridges spend a longer time attached during a cycle than in skeletal muscle

Hill s muscle model consisting of a contractile element (CE) in series with a series elastic element (SE) has been applied to smooth muscle by many investigators (e g Stephens Kroeger & Mehta 1969 Gordon & Siegman 1971 Johansson 1973 Hellstrand & Johansson 1975) The length of the SE and the shortening velocity of the CE depend on the load According to the classical concept a change in load should instantaneously change SE length and CE shortening velocity In skeletal muscle this model is an oversimpli f cation as it has been shown that in well controlled studi s (e g Ford Huxley & Simmons 1977) a major part of the series elasticity is associated w th the cross bridges Moreove a change in load does not instantaneously change shortening elocity to a new st ady level a transitional phase exi ts (Civan & Podolsky 1966) Also in smooth muscle research the simple H ll model has bee econs dered The change in shorte ing velocity following load change is not instantaneous (III H llstrand & Johansson 1979) An t rmediate phase xists characterized by fast shorte ing Not unt l about 75 ms after th quick l as is the length record superimposabl an fterloaded soton c shortening ith th same load The ntermed ate phas lasts for a long t than the one descr bed fo skeletal muscle It seems to be ssociated th cross bridge events as d fferent modes of a ti ation(K a AC stimul tion) affect V_{ma} and the

rate constant of this transient to about the same extent (V) It has also been shown that V_{max} and the rate constant both decrease when temperature is lowered (Hellstrand & Johansson 1979)

The force-velocity relation for bladder muscle is well approximated by Hill s equation V_{max} increases with increasing muscle length to the same extent as isometric force (P_0) in a major part of the rising phase of the length tension relation At lengths near those for optimal force V_{max} increases more than active P_0 This might indicate that the dynamic constants in Hill s equation change but it is equally possible that the force carried by the contractile machinery becomes smaller due to the increasing passive tension at these lengths

V_{max} is lower for K contractures than for AC stimulations giving the same active force (paper V) The reason for the lower V_{max} for tonically contracting muscle is not known In Xenopus slow fibres (Lannergren 1978) shortening velocity to a given load decreases with tim in K high media The reason for the lower V_{max} in the tonic contractions is not known but it is possible that the myofilament lattice may be affected in KCl (cf Jones Somlyo & Somlyo 1973) or that biochemical changes in the cell (e g change in ATP) may influence cross-bridge cycling rate The SE behaviour is unaffected by mode of stimulation (V) suggesting that its major part is passive A minor part of the SE in bladder muscle has been suggested to reside in the ontractil prot ins or structures associated with them (Hellstrand & Johansson 1979 Hellst and 1979)

Strips from bladders that are hypertrophied due to prolonged distension have th same optimum length fo force de elopment as their controls afte normalization to thei in-situ length Ma imum force is lower in hypertrophied mu le but if correction is made for the increased amount of te i these prepa at on the diff rence disappears The hypertrophied muscle cannot horten t the same e tent hen unloaded as th ontrols It is not known if this is due to changes in relations bet een th cells or to changes th th cells The latter possibility seems the most probabl one s cell length in intestinal muscle does not chang he th muscl hype trophies (Gabell 1979a)

6 SUMMARY

Mechanical characteristics of smooth muscle from rabbit and guinea-pig urinary bladder were examined in relation to bladder morphology Muscle cell length (determined by nitric acid maceration or morphometry on fixed tissue) was directly proportional to bladder radius (or stretch of isolated bladder strips) indicating a series coupling of the cells The number of cross sectioned cells per mm^2 varied between 35000 and 170000 and was found to increase in proportion to bladder radius indicating that cell volume is not affected by passive length changes Length-tension (L T) relations were determined for rabbit bladder strips Active force was 19 N/cm^2 at optimum length The strips could shorten actively to 17 % of this length when unloaded During the major portion of the rising part of the L T curve isometric and isotonic L T relations were identical At greater lengths the strips failed to shorten to the same length as when contracting from a shorter starting length L-T relations for a single pig bladder cell were calculated Max active tension at optimum length (400 μm) was 5 5 μN/cell corresponding to 59 N/cm^2 pure muscle Isotonic quick releases were performed on rabbit bladder strips The immediate series elastic (SE) recoil was followed by a transient phase (75 ms) of rapid shortening and finally a steady shortening Max SE recoil was 4 % SE characteristics were unaffected by mode of stimulation suggesting that it is predominantly passive Vmax was 0 37 l/s and 0 26 l/s when strips were activated by AC and K^+ high media respectively The rate constant for the transient was lower for K activation than for AC indicating that it is due to events associated with the contractile proteins Hypertrophic bladder muscle had lower max active tension than controls but the difference disappeared after correction for higher water content in the former Hypertrophic bladders could not shorten to the same ultimate length when unloaded as their controls

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ACTA PHYSIOLOGICA SCANDINAVICA
SUPPLEMENTUM 484

UTERINE SECRETOMOTOR INNERVATION

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UTERINE SECRETOMOTOR INNERVATION

By

MARGARETA HAMMARSTRÖM

STOCKHOLM 1980

Apart from some previously unpublished results, the present thesis is based on the following papers, which are being referred to by their roman numerals.

I. Hammarström, M & Sjöstrand, N O 1979 Evidence for a cholinergic secretory innervation of the guinea pig endometrium. Acta Physiol Scand 106 11—15

II Hammarström, M & Sjöstrand N O 1980 Acetylcholinesterase positive nerves in the guinea-pig endometrium Acta Physiol Scand (In Press)

III Hammarström, M 1980 Endometrial cholinergic secretory responses during estrous cycle, pregnancy and after estrogen and/or progesterone treatment of the guinea pig. Acta Physiol Scand (In Press)

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CONTENTS

I. INTRODUCTION

1. Historical survey

Uterine innervation has been the subject of many comprehensive studies and surveys. Detailed accounts of the early literature are given by e.g. Lee (1841 1858) Frankenhäuser (1867), Labhardt (1906) Hellerman (1927) and Krantz (1959) The reviews of Labhardt (1906), Dahl (1916, 1924) Mabuchi (1924) Hellerman (1927) Davis (1933) and Gruber (1933) present thourough surveys of the work performed on the uterine innervation at the turn of the century Good accounts of literature on research on uterine innervation during recent decades are to be found in the texts by e.g. Reynolds (1949 1965) Krantz (1959) Marshall (1970) Bell (1972) Owman, Sjöberg & Sjöstrand (1974) Thorbert (1978)

However most reviews on uterine innervation reveal that the main interest has been focused on efferent myometrial or vascular innervation or sensory innervation. The possible efferent innervation of the endometrium has attracted considerable less morphological interest and from a physiological point of view virtually no interest. The existence of nerves to the human mucosa of the have even been denied by e.g. Mabuchi (1924) and Schröder (1930)

Yet endometrial nerves have been described in many histological studies. Those first demonstrating the penetration of nerves to the endometrium of mammals using the light microscope appear to be the studies of Kilian (1841) and Frankenhäuser (1867), the latter solely describing nerves to the cervical part of the mucosa.

With the development of metal impregnation techniques and the introduction of supra- and intravital methylene blue staining further and more detailed studies appeared Nerves to the epithelial and glandular layers of the human endometrium were described by Patenko (1880) and Gawronsky (1894). Köstlin (1894) also using silver impregnation confirmed the existence of such nerves in the endometrium of several pecies including the guinea pig. Gentes (1902) using intravital methylene blue staining was able to demonstrate nerves to the epithelial and glandular linings of the endometrium of the rabbit and rat and Labhardt (1906) using both intravital methylene blue staining and the Golgi method demonstrated nerves to the submucosa of the rabbit and human endometrium. Supravital methylene blue staining was used by Hoogkamer (1913) who in various species e g. rabbit, cat, cattle and human was able to demonstrate nerves to the epithelium. Using the same method, Dahl (1916, 1924) observed nerves to the mucosa of the human uterus although he did not pay any greater attention to them. Mabuchi (1924) and Davis (1933) using silver impregnation techniques and intravital methylene blue staining respectively could only demonstrate nerves to the cervical part of the human mucosa. Nerves to the endometrium of the cat were demonstrated by Feldmann (1935) He also noted that part of the endometrial nerves degenerated after hypogastric nerve section.

Several later authors using various metal impregnation techniques have demonstrated nerves to the human endometrium. Thus nerves to the basal third of the endometrium of the adult uterus were observed by State & Hirsch (1941) Freeman (1946) Koppen (1950) and Krantz (1959). Jabonero (1953) noted nerves to the basal two thirds while nerves to the glandular and epithelial linings of the human adult uterus were seen by e.g. Nishumura (1954) and Moricard & Giro (1958) Similar findings were made on the fetal human uterus (Casaglia & Giro 1963) and on the newborn human uterus (Pribor 1951) Nerves to the submucosa of the corpus and a subepithelial plexus in the cervix of the fetal human uterus were also reported by Brown & Hirsch (1941) using the Masson trichrome stain.

Also in several other species nerves to the glandular and epithelial linings of the endometrium have been demonstrated during the last decades as e g in the rabbit (Coujard 1951 Moricard & Giro 1958) and in the rat (Moricard & Giro 1958) as well as in the rhesus macaque (Jacobson & Nieves 1961 Lassman 1965) Nerves to the guinea pig endometrium were denied by Moricard & Giro (1958) while Jacobson & Nieves (1961) demonstrated such nerves with intravital methylene blue staining, thus confirming the statements of Köstlin (1894)

Recent advance in histochemistry has provided more selective nerve staining techniques as e.g. the Falck Hillarp method for adrenergic nerves and the Koelle-Holmstedt method for acetylcholinesterase positive nerves. Thus, Coupland (1962, 1969) demonstrated a rich supply of acetylcholine sterase positive nerves to the human cervical mucosa and a similar but sparser supply to the mucosa of the corpus. An acetylcholinesterase positive plexus spreading all over the rat endometrium was studied in detail by Adham & Schenk (1969). This plexus was accompanied by a scanty adrenergic plexus situated in the basal endometrium. Adrenergic nerves to the basal part of the endometrium were also observed in the rabbit (Owman & Sjöberg 1966), in the rat (Adham & Schenk 1969), in the human (Dallenbach & Vonderlin 1973), and in the guinea-pig (Thorbert et al. 1977).

Using electron microscopy Kühnel & Beier (1976) recently described nerve terminals containing synaptic vesicles to the glandular cells of the endometrium of several mammalian species. Regions of close apposition between nerve terminals, partly losing their Schwann sheath, and glandular cells were observed. In many cases the basal lamina was lacking and the cleft was only about 20 nm suggesting an intimat neuroeffector arrangement.

As evident from this survey nerves to the secretory linings have been repeatedly observed during more than a century. Besides sensory function, a secretomotor function of these nerves also has been postulated by several authors e.g. Schofield (1952), Marshall (1970), Dallenbach & Vonderlin (1973) and Kühnel & Beier (1976). However no study of a possible secretomotor function of these nerves seems to have been made. The absence of such studies is probably due to the difficulty in estimating changes in uterine mucus caused by e.g. nerve stimulation since the amount secreted is very small, viscous and hard to collect. Furthermore in in vivo experiments, there is also a problem in distinguishing active secretion of a small amount of sticky uterine fluid from passive squeezing out of the fluid due to myometrial contraction when uterine nerves are stimulated. In addition to this a secretory response to nerve stimulation might be jeopardized by a concomitant circulatory effect because vasoconstrictor and dilator fibres run among other uterine nerves.

In order to minimize these problems and to standardize the conditions the present study was performed on isolated everted uterine horns. Carbohydrate was used as marker of secretion. The guinea-pig was chosen as experimental animal for the following reasons. a) the anatomy of the uterus and the nerves is clear and easy accessible for experimental conditions. b) It has a reproductive cycle reminding of the human (Thorburn, Challis & Robinson 1977). c) Many basic facts of the uterine innervation and its organization are well settled.

2. Anatomical survey (Fig. 1)

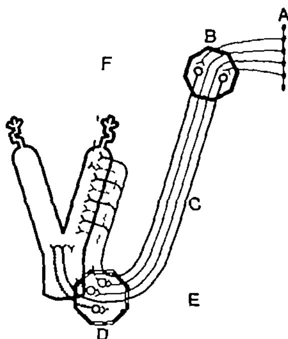


Fig. 1. Schematic drawing of the guinea-pig uterus and its innervation. A. Thoraco-lumbar sympathetic chain. B. Inferior mesenteric ganglion. C. Hypogastric nerve. Part of the fibres in the hypogastric nerve are preganglionic and part of them are postganglionic, the latter relaying in the inferior mesenteric ganglion. D. Paracervical ganglion through which most of the nerves to the uterus pass. Part of the sympathetic supply to the uterus relay in this ganglion. E. A possible uterine innervation via the pelvic nerve has been debated. If present, the fibres are supposed to relay in the paracervical ganglion (D). F. Nerves via the cotoouterine connexion to the uterus: adrenergic nerves are supposed to supply the myometrium of the tubal part of the uterine horn. (Modified ... 1972 and Thorbert 1978.)

The guinea pig has a bicornuate uterus. The uterine nervous supply is derived via two main pathways a) the paracervical ganglia and b) the costouterine connexion (the suspensory ligament) (cf. Gabella 1976, Thorbert et al. 1977)

a) The two paracervical ganglia receive nerves from each hypogastric nerve and also from each pelvic nerve (see below) The hypogastric nerve descends from the inferior mesentric ganglion which receives fibres from the thoraco-lumbar sympathetic chain. According to Thorbert (1978) one half of the adrenergic innervation reaching the uterus via the paracervical ganglia consists of short postganglionic neurons.

The pelvic nerve supply to the uterus via the paracervical ganglia of various mammals has been debated. With respect to the myomotor supply some authors have stated that there is no such supply at all (Langley & Anderson 1896a, vide e.g. Sjöberg 1967 Theobald 1973) Schofield (1952) noted an excitatory effect on the rabbit myometrium in one fourth of experiments on stimulating the pelvic nerve. Other statements are that there is an excitatory myomotor supply (Kör ner 1865 Basch & Hofmann 1877 Fellner 1887 Shabanah, Toth & Maughan 1964 Carsten 1968) or an inhibitory myomotor supply (Dahl 1916, 1924) Basch & Hofmann (1877) and Bell (1972) suggested a cholinergic vasodilator innervation via pelvic nerve of the guinea-pig, however this has not been proved

b) The adrenergic uterine nervous supply via the costouterine connexion mainly reaches the tubal part of the horn of the guinea pig (Thorbert et al 1977) These nerves consist of long post ganglionic fibres and might be derived from the aortico-renal plexus (Kuntz 1947 Thorbert et al. 1977)

3. The reproductive cycle (Fig. 2 & 3)

The adult female guinea pig has an estrous cycle of 16 days duration. The cycle can be roughly divided into *proestrus* lasting for 1—1.5 days, *estrus* often nocturnal, lasting for 7—24 hours, *metestrus* with a duration of —4 days followed by *diestrus* (cf Fig. 2) (Stockard & Papanicolaou 1917 1919 Ishii 1928, Young 1936/37 Burgos & Wislocki 1956, 1958)

A rough estimate of the occurrence of the estrus phase can be made by inspection. Thus the vaginal closure membrane is open for 2—4 days during estrus. From the appearance of vaginal smears the estrus phase could be further divided. This renders the possibility to better determine the time of ovulation (vide Stockard & Papanicolaou 1917 1919 Selle 1922, Young 1936/37 Bland & Donovan 1970) The day of ovulation is designed day 1 of the cycle.

If conception occurs, the ovum reaches the uterus at the end of day 3 or the beginning of day 4 (Bishoff 1852, Squier 1932) Implantation occurs on day 6—7 (Bishoff 1852, von Spee 1901 Emrys-Roberts 1909/10, Sansom & Hill 1931 and Blandau 1949). The gestational time is 63—68 days (Bishoff 1852, Phoenix 1970) 1—4 hours post partum the para gets into estrus (Bishoff 1852, Ishii 1928, Boling et al 1939 Phoenix 1970) She ovulates and conceives if fertilized.

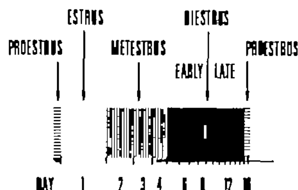


Fig 2 Schematic illustration showing the estrous cycle of the guinea-pig Ovulation occurs at day one. (Days on logarithmic scale.)

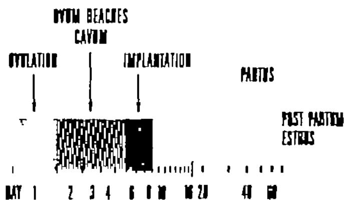


Fig. 3. Schematic illustration of the sequences of pregnancy of the guinea-pig. (Days on logarithmic scale.)

II MATERIAL AND METHODS

Animals. Sexually mature mottled or albino virgin guinea pigs (300—500 g) were used

Hormonal treatment In paper I estradiol benzoate 6 mg/kg in combination with hydroxyprogesterone caproate 150 mg/kg (Primosiston®) was injected every second day for 10—14 days. In paper III estradiol valerate 6 mg/kg (Progynon®) and hydroxyprogesterone caproate 150 mg/kg (Proluton®) alone or in combination was given every second day for 10—14 days. In paper II estradiol benzoate 6 mg/kg in combination with hydroxyprogesterone caproate 150 mg/kg (Primosiston®) were injected every third day for 6—8 days.

Estrous cycle determination (III) The animals were controlled daily concerning the status of the vaginal closure membrane. Only animals showing regular cycles were chosen. After at least two normal cycles, vaginal smears were taken when the membrane was open The day of maximum cornification before leucocytic influx was designated day I (cf Stockard & Papanicolaou 1917 1919 Bland & Donovan 1970 Thorbert 1978)

Determination of pregnancy (III) 4—5 females were caged with one male. The females were con- l daily concerning the status of the vaginal closure membrane. Pregnancy was estimated from the second day of opening and controlled by palpation early in pregnancy

Anaesthesia. All operations were performed under sodium pentobarbital anaesthesia when necessary supplemented with ether (I & III)

Operative procedures. Bilateral denervations were undertaken 10—1 days before an experiment was performed (I) The *hypogastric erve* was sectioned 1 cm below the inferior mesenteric ganglion and about 2 mm of the nerve was removed Each *pelvic nerve* was similarly sectioned 1 cm proximal to its crossing of the iliac artery The *paracervical ganglia* were destroyed with phenol (8.5 M) and the nerves of the *costouterine connexion* were destroyed by a tight ligature dipped in 8.5 M phenol.

Bilateral *oophorectomi* (III) was performed via dorsal incisions 3 weeks before hormone treatment was started.

Preparation (dissecting and mounting procedures) (cf paper I) All animals were stunned and bled the uterus and parametrial tissue were dissected out. The uterus was divided through the cervix, each horn everted and mounted on a platinum electrode. Each horn was put in an organ bath of 25 ml or 100 ml (late pregnancy horns) containing Tyrode solution (Fig. 4)

Many factors contributed to the fact that the whole uterus was used. The innervation is richest

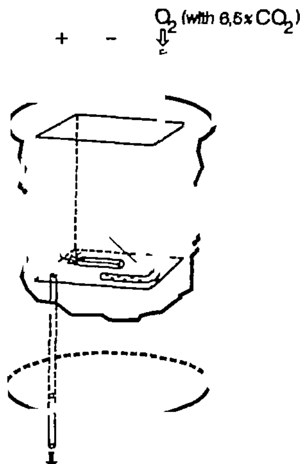


Fig 4 Schematic drawing of experimental set-up. Tyrode solution (36 C) is poured into the organ bath from above White arrow shows tube for carbogen gas supply The everted uterine horn is mounted on an pl t num electrode, shielded except for the part situated within the uterine horn The electrode is supplied with a small glass knob in order to avoid penetration of the uterine wall The other electrode is isolated except for its last part. Black arrow indicates the outlet for Tyrode solution.

in the cervical part of the mucosa of human and rat (Coupland 1962, 1969 Adham & Schenk 1969). The cervix alone is however impossible to evert and it is difficult to identify by eye the limit of the cervical part of the mucosa which might render an insecurity concerning preparation from experiment to experiment.

Experimental performance Experiments were performed following the schedule given in paper I.

After 80 min accomodation in ordinary Tyrode solution changed every 20 min, the sampling was started. Five 10 min sampling periods were performed in Tyrode solution where glucose was replaced by NaCl. The sampling periods were separated by resting periods of 20 min in ordinary Tyrode solution. The first, third and fifth sampling periods were used for determination of carbohydrate resting secretion level. The second and fourth periods were used for studying the effect of nerve field stimulation or administration of drugs, or both. In certain experiments only three sampling periods were performed. In these cases there were two sampling periods to determine resting secretion level surrounding one stimulation period.

Estimation of secretion. Choice of marker and method As the endometrium undergoes cyclical changes a marker not affected by such changes is hardly possible to find. Since the uterine mucus is rich in carbohydrates of many different types, it was necessary to use a method for determination of total carbohydrate amounts. The method chosen was the indole-sulfuric acid method for total carbohydrates (Dishe & Popper 1926) used for determination of total carbohydrate amount in cervical mucus by Shettles (1951) and Shettles, Dishe & Osnos (1951)

Analysis of samples. The volumes from the sampling periods were lyophylized (I II) or evapourated (at 40 C) (late pregnancy experiments, III) The dry residue from the sampling periods was dissolved in 0.5 ml (1 ml) water and 4.5 ml (9 ml) 14 4 M H_2SO The samples were centrifuged and the supernatant (3 ml or 4.5 ml) was collected. To it was added 0 10 ml (0.15 ml) 8.5×10^{-3}M indole (figures between brackets for samples from experiments late in pregnancy). The sample was then heated for 10 min in 100 C. The carbohydrate concentration was determined spectrofotometrically (470 nm) using an internal standard.

Determination of mucosal carbohydrate content After the experiment the endometrial layer was separated from the myometrium and a small piece was taken for analysis. Each piece was homogenized by grinding with quarts sand in a mortar containing 0.5 ml of 14 4 M H_2SO_4 Rinsing was performed with 4 ml 14 4 M H_2SO_4 and 0.5 ml distilled water After shaking the homogenate was centrifuged. 4.5 ml of the supernatant was removed and to it 0.15 ml 8.5×10^{-3}M indole was added and the sample analysed as described above.

Stimulations Field stimulation of nerves was applied at a standard rate of 0.5 Hz, 0.5 ms, 30 V Nerve stimulation was applied with a frequency of 5 Hz (0 1 ms, 30 V) for 10 s every 30 s. Carbachol was administrated in doses of 5.5×10^{-10}—5.5×10^{-5}M.

Calculation and statistics Because of the rather great variance between different experiments in resting level of carbohydrate secretion all results were calculated as a percent of resting secretion level using the mean of the three (two) resting periods as matching value. Each type of experiment was performed on uteri from at least three animals.

The Students t-test or when necessary the Welch approximative t test was used for statistical comparison of experiments (Remington & Schork 1970)

Drugs Apart from the hormones obtained from Schering AG drugs used were: carbamylcholine chloride (Carbachol) (Sigma) scopolamine hydrobromide (Sigma) phentolamine chloride (Ciba), sotalol hydrochloride (MJ 1999 Regis Chemical Company) isoprenaline sulphate (Abbot), methoxamine hydrochloride (BDH), tetrodotoxin (TTX) (Sigma)

Tyrode solution composition NaCl 13.7×10^{-2} KCl 2.7×10^{-3} MgCl 4.9×10 $CaCl_2$ 1.8×10^{-3} $NaHCO_3$ 1.2×10^{-2} Na_2HPO $2H_2O$ 3.1×10^{-4}M 1.1×10^{-2}mol of glucose was added/l of ordinary Tyrode solution and 5.6×10^{-2}mol of NaCl to each liter of glucose free Tyrode solution.

Demonstration of acetylcholinesterase positive nerves (II) The modified Koelle Holmstedt method of Karnovsky & Roots (1964) was used for identifying AChE positive nerves. The uterus was carefully dissected out and cut into 5—8 mm pieces. The pieces were either directly frozen (—30 C) or frozen after 1—16 h of fixation in ice-cold 10 % formalin containing 1 —

Uvnäs 1960 Sjöqvist 1963 Folkow & Neil 1971) A secretory innervation of vaginal glands via the hypogastric nerve has been suggested by Davis (1934) Furthermore, the secretory innervation of the dog prostate is cholinergic and sympathetic. Most fibres seem to be preganglionic and run in the hypogastric nerve (vide Sjöstrand 1976) Also cholinergic vasodilator fibres to the penis of rabbit seem to have a preganglionic supply via the hypogastric nerve (Sjöstrand & Klinge 1979)

Secretomotor innervation via the pelvic nerve and/or the costouterine connexion cannot be ex cluded. However possible secretomotor fibres passing these routes can hardly contribute to a very prominent proportion of the secretomotor in nervation. Here it is to be mentioned, that ac cording to Thorbert et al. (1977) no acetylchol inesterase positive nerves are present in the cost outerine connexion. A possible uterine innervation derived from the pelvic nerve is subject to dif ferent opinions (see Introduction)

The above statements concerning pathways and synaptic relays of the secretomotor fibres are guarded and not too precise. This must be so be cause there is not only the roughness of the me thod to be considered Also possible phenomena as denervation supersensitivity of effector cells deprived of their original innervation but excited by minute amounts of diffused transmitter and reinnervation of effector cells from sprouting terminals deriving from unsevered nerves have to be taken into account

2. Secretory responses to autonomic drugs (I)

The results documented above led to the conclusion that uterine secretory responses were evoked by stimulation of cholinergic nerves having their main course—pre- or postganglionic—in the hypogastric nerve. The receptors on the secretory cells were apparently muscarinic

Accordingly it was considered to be of interest to study the effect of a muscarinic drug. Carbachol was chosen because of its high potency and resistance to acetylcholinesterase *Furthermore, in* the extended study (see below) transmural stimulation of nerves was impossible to accomplish in pregnancy (day 16—42) which made experiments with a muscarinic drug necessary

Carbachol (5.5×10 M) increased secretion to the same extent as did transmural stimulation, the response was dose dependent with a sharp threshold at 5.5×10^{-8}M whereafter maximum rapidly occurred. *Scopolamine* (1.3×10^{-6}M) abolished the effect of carbachol. This further substantiated the muscarinic nature of the excitatory receptors of the secretory cells and is in line with the studies of Marco (1930) Shih, Kennedy & Huggins (1940) and Kaulla et al (1957) which demonstrated alterations in uterine secretion after pilocarpin administration to bitch or woman.

Neither the α-adrenoceptor stimulant methoxamine (4×10^{-8}M 4×10^{-4}M) nor the β-adrenoceptor agonist isoprenaline (2×10^{-6}M) produced any clearcut effects on endometrial secretion. Consequently no evidence of any significant adrenergic mechanism affecting uterine secretion was obtained in these experiments.

3. Alterations in secretory responses after female sex hormone treatment, in estrous cycle and during pregnancy (I III)

The experiments described above elucidating endometrial innervation and its sources were per formed on animals purposely brought into arti ficial secretory phase For natural reasons it appeared to be of interest to investigate possible secretory responses during normal reproductive cycle of the guinea pig and after excess of estrogen and/or progesterone treatment.

Combined t eatment with estrogen and progest rone a) *Intact animals* (I) treated with the commercial product Primosiston® *Field stimulation* of nerves produced a secretory response of +71 % *Carbachol* ($5.5\times10^{-}$ M) raised the secretion to the same level (+68 %) Threshold concentration was 5.5×10^{-8}M (see above)

b) Spayed animals (III) treated with the commercial products Progynon ® (estrogen) and Proluton ® (progesterone) *Transmural stimulation of nerves* ncreased secretion by 56 % *Carbachol* produced an increase in secretion by about the *same magnitude* (+41 % *at* 5.5×10^{-8}*M* and +49 % at 5.5×10^{-7}M) as did transmural stimulation of nerves. The sensitivity to carbachol was similar to that of the intact animals treated w th both hormones. (The differences between these two groups in degree of response to transmural stimulation or to carbachol are not statistically significant.)

in the cervical part of the mucosa of human and rat (Coupland 1962, 1969 Adham & Schenk 1969) The cervix alone is however impossible to evert and it is difficult to identify by eye the limit of the cervical part of the mucosa which might render an insecurity concerning preparation from experiment to experiment.

Experimental performance Experiments were performed following the schedule given in paper I.

After 80 min accomodation in ordinary Tyrode solution changed every 20 min, the sampling was started. Five 10 min sampling periods were performed in Tyrode solution where glucose was replaced by NaCl. The sampling periods were separated by resting periods of 20 min in ordinary Tyrode solution. The first, third and fifth sampling periods were used for determination of carbohydrate resting secretion level. The second and fourth periods were used for studying the effect of nerve field stimulation or administration of drugs, or both. In certain experiments only three sampling periods were performed In these cases there were two sampling periods to determine resting secretion level surrounding one stimulation period.

Estimation of secretion Choice of marker and method As the endometrium undergoes cyclical changes a marker not affected by such changes is hardly possible to find. Since the uterine mucus is rich in carbohydrates of many different types, it was necessary to use a method for determination of total carbohydrate amounts. The method chosen was the indole-sulfuric acid method for total carbohydrates (Dishe & Popper 1926) used for determination of total carbohydrate amount in cervical mucus by Shettles (1951) and Shettles, Dishe & Osnos (1951).

Analysis of samples. The volumes from the sampling periods were lyophylized (I III) or evapourated (at 40 C) (late pregnancy experiments, III) The dry residue from the sampling periods was dissolved in 0 5 ml (1 ml) water and 4.5 ml (9 ml) 14 4 M H_2SO_4 The samples were centrifuged and the supernatant (3 ml or 4.5 ml) was collected To it was added 0 10 ml (0 15 ml) 8.5×10^{-3}M indole (figures between brackets for samples from experiments late in pregnancy). The sample was then heated for 10 min in 100° C. The carbohydrate concentration was determined spectrofotometrically (470 nm) using an internal standard

Determination of mucosal carbohydrate content After the experiment the endometrial layer was separated from the myometrium and a small piece was taken for analysis. Each piece was homogenized by grinding with quarts sand in a mortar containing 0.5 ml of 14 4 M H_2SO_4 Rinsing was performed with 4 ml 14 4 M H_2SO_4 and 0.5 ml distilled water After shaking the homogenate was centrifuged 4.5 ml of the supernatant was removed and to it 0 15 ml 8.5×10^{-3}M indole was added and the sample analysed as described above.

Stimulations. Field stimulation of nerves was applied at a standard rate of 0.5 Hz, 0.5 ms, 30 V Nerve stimulation was applied with a frequency of 5 Hz (0 1 ms, 30 V) for 10 s every 30 s. Carbachol was administrated in doses of 5.5×10^{-14}—5.5×10^{-8}M

Calculation and statistics Because of the rather great variance between different experiments in resting level of carbohydrate secretion all results were calculated as a percent of resting secretion level using the mean of the three (two) resting periods as matching value. Each type of experiment was performed on uteri from at least three animals.

The Students t-test or when necessary the Welch approximative t-test was used for statistical comparison of experiments (Remington & Schork 1970)

Drugs Apart from the hormones obtained from Schering AG drugs used were, carbamylcholine chloride (Carbachol) (Sigma) scopolamine hydrobromide (Sigma) phentolamine chloride (Ciba) sotalol hydrochloride (MJ 1999 Regis Chemical Company), isoprenaline sulphate (Abbot), methoxamine hydrochloride (BDH), tetrodotoxin (TTX) (Sigma)

Tyrode solution composition NaCl $13\,7\times10^{-2}$ KCl 2.7×10^{-3} $MgCl_2$ $4\,9\times10^{-4}$ $CaCl_2$ 1.8×10^{-3} $NaHCO_3$ 1.2×10^{-2} Na_2HPO $2H_2O$ 3.1×10^{-4}M, $1\,1\times10$ mol of glucose was added/l of ordinary Tyrode solution and $5\,6\times10^{-3}$mol of NaCl to each liter of glucose free Tyrode solution.

Demonstration of acetylcholinesterase positive nerves (II). The modified Koelle Holmstedt method of Karnovsky & Roots (1964) was used for identifying AChE positive nerves. The uterus was carefully dissected out and cut into 5—8 mm pieces. The pieces were either directly frozen (—30 C) or frozen after 1—16 h of ice-cold 10 % formalin

(Karnovsky & Roots) followed by 1—4 days in ice-cold "gum-sucrose"-solution (0.88 M sucrose containing 1 % gum arabic) (Holt, Hobbiger & Pawan 1960). Some unfixed sections were post fixed in formalin according to El-Badawi & Schenk (1967). Sections (10 μ) were cut in a cryostat. Time in the incubation mediums recommended by Karnovsky & Roots was 1—5 h. Iso-OMPA (4×10^{-4}M) was used to inhibit nonspecific cholinesterase activity. For general staining of the sections eosin or lichtgrün was used.

III. RESULTS COMMENTS AND CONCLUSIONS

1. Secretory nerves to the guinea-pig endometrium (I & II)

The aim of these studies was to collect evidence of a secretomotor function of certain endometrial nerves. Unspayed guinea-pigs, whose endometria had been brought into artificial secretory phase were used (see Material and Methods)

a. Presence and nature of endometrial secretomot innervation

At the standard field stimulation rate an increase in secretion of 70 % was seen. This response was abolished by TTX (3×10^{-7}M) *Scopolamine* (1.3×10^{-6}M) levelled away the secretory response while *sotalol* (1.8×10^{-7}M) was without overt effect After *phentholamin* (1.6×10^{-6}M) the secretory response reached +84 % (This value was not statistically significantly higher than the value obtained after field stimulation solely)

The susceptibility of the field stimulation response to TTX, suggested a neurogenic origin. Since the response was abolished by scopolamine the nerves were likely to be cholinergic and the receptors on the endometrial secretory cells mus carinic. These suggestions were further supported by the demonstration of acetylcholinesterase positive nerves in the guinea-pig endometrium (II) Such nerves have been reported in the human endometrium (Coupland 1962, 1969) and Adham & Schenk (1969) have demonstrated a mucosal acetylcholinesterase positive plexus to the epithelial and glandular layers of the rat endometrium. However some authors have not been able to demonstrate such nerves, in human, rabbit or guinea-pig endometrium (Owman & Sjoberg 1966, Jordan 1970 Thorbert et al. 1977). The diversity in opinion is probably due to difficulties involved in displaying endometrial nerves since it is difficult to obtain a proper fixation attended by a desired staining (cf Theobald 1973, II).

From the results it can be concluded that the guinea-pig endometrium possesses a cholinergic secretory innervation Presumably this conclusion could be extended to other mammals, at least to man and rat.

b Possible origin and course of the cholinergic secretomotor nerves

Stimulation of extrinsic nerves. Stimulation of the hypogastric nerve produced an increase in secretion by about 40 % while pelvic nerve stimulation was without overt effect.

Experiments on denervated uteri Field stimulation after section and degeneration of the *hypogastric nerve* resulted in a secretory response of +26 % i.e. about one-third of the control level. Destruction of *paracervical ganglia* produced a further decrease in the obtained secretory responses to one-sixth (+11 %) Chronic interruption of the *pelvic nerve* as well as of the *costo-uterine connexion* produced no definite reduction in uterine secretory responses (+56 % and +52 % respectively)

Consequently the hypogastric nerve seems to be the main secretomotor pathway Part of the fibres are postganglionic, part of them are preganglionic, the latter relaying in the paracervical ganglia. A similar pattern with pre and postganglionic fibres in the hypogastric nerve has been suggested for uterine myomotor nerves in the cat and rabbit (Langley & Anderson 1896b) in the rabbit (Varagic 1956) and in the guinea-pig (Rüsse & Marshall 1970) Feldmann (1935) showed degeneration of part of the uterine nerves of the cat following section of the hypogastric nerve. Furthermore, this organization is in agreement with studies on distribution of adrenergic terminals and the presence of the adrenergic transmitter noradrenaline, in the myometrium (for ref see Sjöberg 1967 Owman et al. 1974 Thorbert 1978). These studies show that postganglionic adrenergic fibres pass via the paracervical ganglia and that preganglionic fibres relay there, i.e. on short adrenergic neurons. Also cholinergic vasodilator fibres relay in the paracervical ganglia, their preganglionic source being more obscure (Bell 1972, 1974a, b).

From an organizational point of view the uterine secretomotor fibres would be regarded as belonging to the sympathetic system. Cholinergic sympathetic innervation to sweat glands and blood vessels of skeletal muscle of certain species is well documented (vide Langley 1891 1895

Uvnäs 1960 Sjöqvist 1963 Folkow & Neil 1971) A secretory innervation of vaginal glands via the hypogastric nerve has been suggested by Davis (1934) Furthermore, the secretory innervation of the dog prostate is cholinergic and sympathetic. Most fibres seem to be preganglionic and run in the hypogastric nerve (vide Sjöstrand 1976) Also cholinergic vasodilator fibres to the penis of rabbit seem to have a preganglionic supply via the hypogastric nerve (Sjöstrand & Klinge 1979)

Secretomotor innervation via the pelvic nerve and/or the costouterine connexion cannot be excluded. However possible secretomotor fibres passing these routes can hardly contribute to a very prominent proportion of the secretomotor innervation. Here it is to be mentioned, that according to Thorbert et al (1977) no acetylcholinesterase positive nerves are present in the costouterine connexion. A possible uterine innervation derived from the pelvic nerve is subject to different opinions (see Introduction)

The above statements concerning pathways and synaptic relays of the secretomotor fibres are guarded and not too precise. This must be so because there is not only the roughness of the method to be considered Also possible phenomena as denervation supersensitivity of effector cells deprived of their original innervation but excited by minute amounts of diffused transmitter and innervation of effector cells from sprouting ...inals deriving from unsevered nerves have to taken into account.

2. Secretory responses to autonomic drugs (I)

The results documented above led to the conclusion that uterine secretory responses were evoked by stimulation of cholinergic nerves having their main course—pre- or postganglionic—in the hypogastric nerve. The receptors on the secretory cells were apparently muscarinic.

Accordingly it was considered to be of interest to study the effect of a muscarinic drug Carbachol was chosen because of its high potency and resistance to acetylcholinesterase Furthermore, in the extended study (see below) transmural stimulation of nerves was impossible to accomplish in pregnancy (day 16—42), which made experiments with a muscarinic drug necessary

Carbachol (5.5×10 M) increased secretion to the same extent as did transmural stimulation, the response was dose dependent with a sharp threshold at 5.5×10^{-8}M whereafter maximum rapidly occurred. *Scopolamine* (1.3×10^{-6}M) abolished the effect of carbachol This further substantiated the muscarinic nature of the excitatory receptors of the secretory cells and is in line with the studies of Marco (1930) Shih, Kennedy & Huggins (1940) and Kaulla et al (1957) which demonstrated alterations in uterine secretion after pilocarpin administration to bitch or woman

Neither the α-adrenoceptor stimulant methoxamine (4×10^{-8}M 4×10^{-4}M) nor the β adrenoceptor agonist isoprenaline (2×10^{-6}M) produced any clearcut effects on endometrial secretion. Consequently no evidence of any significant adrenergic mechanism affecting uterine secretion was obtained in these experiments.

3. Alterations in secretory responses after female sex hormone treatment, in estrous cycle and during pregnancy (I, III)

The experiments described above elucidating endometrial innervation and its sources were performed on animals purposely brought into artificial secretory phase. For natural reasons it appeared to be of interest to investigate possible secretory responses during normal reproductive cycle of the guinea-pig and after excess of estrogen and/or progesterone treatment

Combined treatment with estrogen and progesterone a) *Intact animals* (I) treated with the commercial product Primosiston® *Field stimulation* of nerves produced a secretory response of +71 % *Carbachol* (5.5×10^{-8}M) raised the secretion to the same level (+68 %) Threshold concentration was 5.5×10^{-8}M (see above)

b) Spayed animals (III) treated with the commercial products Progynon ® (estrogen) and Proluton ® (progesterone) *Transmural stimulation of nerves* increased secretion by 56 % *Carbachol* produced an increase in secretion by about the same magnitude (+41 % at 5.5×10 M and +49 % at 5.5×10^{-7}M) as did transmural stimulation of nerves. The sensitivity to carbachol was similar to that of the intact animals treated with both hormones. (The differences between these two groups in degree of response to transmural stimulation or to carbachol are not statistically significant.)

Single hormone treatment of spayed animals (III). a) *Estrogen. Field stimulation* of nerves resulted in an increase of secretion by 14 %. *Carbachol* (5.5×10^{-7}M) raised the secretion to about the same level as did nerve stimulation (+25 %). The response was shifted to the right by more than the power of ten, compared to the group receiving both estrogen and progesterone.

b) *Progesterone.* Neither *field stimulation of nerves* nor *carbachol* (up to 5.5×10^{-6}M) produced any definite secretory responses in this group.

Estrous cycl (III). Responses to transmural nerve stimulation of about +20 % were noted around estrus (day 16—2) and at early diestrus (day 6—11). At metestrus (day 3—5) an inhibitory answer was obtained after field stimulation of nerves (—18 %). This response was reversed when *phentolamine* (1.6×10^{-6}M) was added (+25 %). However methoxamine (4×10^{-6}M) did not produce any definite effect on the secretion. The response to carbachol (5.5×10^{-8} — 5.5×10^{-6}M) reached about +20 % at day 16—11 i.e. an answer of the same magnitude as that to transmural stimulation of nerves. The secretory responses of uteri from animals in estrous cycle (day 16—11) showed the same sensitivity to carbachol as those brought into artificial secretory phase by combined female sex hormone treatment. The magnitude of the response was however only about one third of the latter.

P egnancy (III). At *impl tation t me* (day 6—8) a response of about +20 % was obtained by *transmural st mul tion of nerves* as well as by *carbachol* (5.5×10 M — 5.5×10^{-6}M). From day 16—42 transmural stimulation was impossible to accomplish since the uterine wall was partly spl t (see M terial and Methods III). At day 16—18 no secretory response to carbachol (5.5×10 M — 5.5×10- M) was obtained. At *mid pregnancy* an increasing response to carbachol (5.5×10^{-6}M — 5.5×10 M) was noted (+30 % at day 28—32, +50 % at day 38—42). In *late* pregnancy the response t carbachol (5.5×10^{-7}M) reached +62 % at day 48—52 and +56 % at day 63—65. The response to transmural stimulation of nerves was of a similar magnitude in late pregnancy as that seen after carbachol. The responses to transmural nerve stim lation and to carbachol of uteri in late pregnancy were of the same degree as those of uteri from animals brought into artificial secretory phase (cf. above). The sensitivity to carbachol seemed however to be somewhat lower. *Post partum* the secretory response (about +50 %) after *transmural nerve stimulation* as well as to carbachol (5.5×10^{-8}M — 5.5×10^{-6}M) persisted.

Apparently carbachol raised the secretion to the same level as did transmural stimulation of nerves, when both stim li were tested. However the magnitude of the response as well as the sensitivity *to carbachol varied with the hormonal state of the animal.* Combined treatment with estrogen and progesterone seems to mimic late pregnancy with respect to the magnitude of the responses. The response in estrous cycle (day 16—11) is smaller (about one third) but the sensitivity to carbachol is similar to that of uteri brought into artificial secretory phase. Uteri from animals treated with estrogen only also showed answers of smaller magnitude but were also less sensitive to carbachol.

Some aspects on these differences have to be mentioned. With respect to the magnitude of the responses one must keep in mind the variation of the resting secretion level. A high resting secretion level might more or less disguise a secretory response (cf. below III).

The sensitivity to carbachol was less in the group receiving estrogen only. Also pregnant uteri tended to be less sensitive to carbachol. From this it can be suggested that changes in the sensitivity of number of and accessibility to receptors can take place according to the hormonal state of organism. The absence of responses to both carbachol and transmural stimulation of nerves in late diestrus and of uteri from animals treated with progesterone only is probably explained by an inability of the endometrial cells to respond to secretory stimuli at least with respect to the parameters studied (see also below).

Obviously both female sex hormones seem to be necessary for development of full secretory effect. Female sex hormones are necessary for the development f the secretory cell i.e. the growth of organelles as mitochondria and Golgi apparatus as well as organization of microtubuli. These organelles seem to be necessary for secretory ability (Themann & Schünke 1963, Dallenbach Hellweg 1969, Lawn 1973, Hafez & Ludwig 1977, Wynn 1977). Furthermore the development of the glands in secretory phase increases the area of mucus producing cells which can affect the cretory response as well as the resting

level Moreover variation in type and amount of carbohydrate secreted may contribute to changes in the secretory responses and also to resting secretion level. Such changes in secretory products occur regularly in the human endometrium during reproductive cycle (Cramer & Klöss 1955 Runge Ebner & Lindenschmidt 1956, Schmidt Matthiesen 1963) In this context it should be recalled that endometrial secretion seems to be of the apocrine type (vide e.g. Wetzstein & Wagner 1960 Schmidt Matthiesen 1963 Hafez & Ludwig 1977) This type of secretion might be the reason for the all or none answer to secretory stimuli (cf I)

In this context the serum levels of estrogen and progesterone in the reproductive cycle of the guinea pig should be mentioned. Concerning progesterone, the serum concentration is lowest during late diestrus (day 11—15) and reaches its peak in metestrus and early diestrus (day 5—9) The estrogen levels cannot be adequate measured during the estrous cycle (Feder Resko & Goy 1968 Challis, Heap & Illingworth 1971 Croix & Franchimont 1975) In mid and late pregnancy the progesterone concentration is 100-folded increased compared to the peak in estrous cycle. The estrogen levels increase from mid to late pregnancy about 3-fold (Heap & Deansley 1966, Challis et al 1971) Accordingly the combined treatment with estrogen and progesterone chosen in this study seems to mimic the hormonal state at late pregnancy

Possible alternative or contributive reasons for the variations in secretory responses could be hormonally caused changes in the neuroeffector region such as alterations in nerve fibre density in transmittor content of nerve terminals, in size of junctional cleft and in the sensitivity number and distribution of postjunctional or maybe, also prejunctional receptors. Thus t has been reported that mucosal acetylcholinesterase positive nerves are more numerous and more intensely stained at estrus in the rat (Adham & Schenk 1969) It has also been suggested that endometrial nerves of the macaque degenerate at menstruation (Jacobson & Nieves 1961)

Since the magnitude of the responses to nerve stimulation and carbachol respectively essentially were parallel in all groups where secretory responses were obtained, and both stimuli could be applied, it seems unlikely that changes at least in the neural part of the region contributed very much to the observed differences in size of responses. However concerning the absence of responses of uteri in late diestrus or dominated by progesterone only alterations as e.g. paucity of postjunctional receptors and degeneration of nerves might have some significance.

The results obtained with field stimulation at day 3—5 in estrous cycle indicated an α-adrenergic, possibly neurogenic, inhibition of the secretory response This inhibition could be pre or post junctional or both. The absence of a clear-cut effect of the exogenous α-adrenoceptor agonist methoxamine (I III) on uterine secretion would suggest a prejunctional inhibition (Paton & Vizi 1969) (vide Burnstock & Costa 1975 Stjärne 1977) However one must also take possible diffusion barriers to exogenous agonists created e.g. by the outer layers of the endometrium into consideration. Therefore, at present, it is impossible to definitely point out the localization of the adrenergic inhibition. Adrenergic nerves in the endometrium are essentially localized in the basal endometrium (see Introduction) Also this localization might suggest a prejunctional site of action of these nerves on cholinergic secretomotor innervation, The fact that clear evidence of an adrenergic counteraction of the cholinergic response to field stimulation was noted only at day 3—5 in estrous cycle could be due to the circumstance that adrenergic innervation and noradrenaline content in the guinea-pig uterus are hormone dependent (vide III) It should however also be kept in mind that changes in stimulation frequencies might have revealed such an influence in other preparations too

A further conclusion, possibly quite significant, can be drawn from the results i.e. there does not seem to be any disappearance of the cholinergic secretomotor innervation during pregnancy This is in line with the results of Thorbert et al (1979) showing that cholineacetyltransferase levels are not influenced during pregnancy The statement made above concerning cholinergic endometrial innervation contrast to what happens to the myometrial adrenergic innervation during pregnancy which disappears (cf Owman et al 1974 Thorbert 1978) Also combined treatment with estrogen and progesterone reduces uterine noradrenaline content (Thorbert et al. 1978b). The largest secretory responses to nerve stimulation were noted in uteri of animals receiving huge doses of these

hormones or being in late pregnancy. The neurogenic cholinergic secretory responses persisted post partum while still three months post partum it is difficult to descern adrenergic terminals (Thorbert et al. 1978a).

4. Resting secretion level and endometrial carbohydrate concentration (I & III)

In this section the resting secretion levels of the different groups will be compared and commented upon. Since secretion level might depend on carbohydrate concentration of the mucosa, this will also be commented.

Resting secretion level per 10 min was rather uniform throughout all series with maximum occurring at pregnancy day 16—18 and in late pregnancy (day 63—68). The lowest values were seen in the groups receiving combined hormone treatment (0.34 ± 0.14 μmol n=49 (I) and 0.47 ± 0.17 μmol n=21 (III)).

As mentioned above and in paper III a high resting secretion level might disguise a possible secretory response e.g. at pregnancy day 16—18. This possibility should also be considered in the case of lack of secretory responses in late diestrus and in the group treated with progesterone only. In estrous cycle the resting secretion level did not differ statistically between the groups ($p > 0.05$) but the levels were always high (0.61 — 0.85 μmol) (cf. Table 1 in paper III) as they were in the estrogen treated group (0.72 μmol and in the progesterone treated group (0.60 μmol) (cf. Table III in paper III). Since secretory responses were noted in estrous cycle (day 16—11) and in the estrogen treated group despite high resting secretion levels it seems unlikely that the inability to respond to secretory stimuli of uteri from the progesterone treated group and of uteri in late diestrus phase merely is an expression of a high resting secretion level masking a possible secretory response.

Carbohydrate leakage from all types of uterine tissue makes however the evaluation of secretory responses somewhat uncertain. For natural reasons the uncertainty is most marked when small secretory responses and small differences in secretory responses between groups are evaluated. This is one reason why one cannot make precise statements on e.g. the effect of methoxamine (I, III) phentolamine (I) and interruption of pelvic nerve or costouterine connexion (I).

Throughout the series endometrial carbohydrate concentration was determined in order to obtain information of conceivable changes in carbohydrate concentration due to the hormonal state of the uterus (value for experiments in paper I 0.34 ± 0.13 μmol/g n=49 (previously unpublished) for others see III). However the endometrial carbohydrate concentration was rather uniform in all the series with maximum occurring in pregnancy day 16—18 and in late pregnancy (day 63—68) (III). This conformity is probably due to the circumstance that female sex hormones rather affect certain types of carbohydrates and their relative distribution in the glandular epithelial and stromal layers of the endometrium than change the total carbohydrate concentration (Schmidt-Matthiesen 1963, III).

IV GENERAL DISCUSSION

Uterine secretion and especially cervical secretion and the role of the mucus in mammalian reproduction have been subject to great interest for many years. Since now in addition to earlier mor phological studies evidence exists of a functional secretomotor innervation of the endometrium of one species (the guinea pig) some brief speculations on the function of this innervation seem to be justified. Uterine innervation has been claimed to be unsignificant since spinal cord lesions and section of the hypogastric or presacral" nerve and the pelvic nerve of various species e.g. rabbit, dog and man do not seem to create an obstacle for fertilization, fetal growth and delivery in individual cases (Goltz & Freusberg 1874 Rein 1880 Gerstmann 1926, Bacq & Brouha 1932, Wetherell 1935 Donaldson 1936, Cotte 1937 Pearce 1940, Blinick 1947 Newell & Smithwick 1947 Poppen & Lemmon 1947 Guttman 1964 Jackson 1964 Hardy & Warrell 1965) However *about half the uterine innervation is derived from* short neurons, the cell bodies of which are located in the paracervical ganglia. This implies that at least the possible trophic effects of the nerves remain, after the above mentioned lesions Fur thermore, a controlled study on the effects of denervation on a large group of individuals has not been performed there are only case re ports.

Female sex hormones constitute the necessary basis for uterine function and as far as uterine secretion is concerned the hormones cause the development of the secretory cells. This development is essential for the secretion of mucus from the cells (cf. Chapter III) The nerves may then act as fine modulators on the secretory cell.

The significance of the nerves could either be merely trophic or directly secretomotor which the present results suggest. Discharges in secretomotor nerves might arise on a purely reflexogenic basis, as a result of sensations due to external or internal stimuli, due to adjustments in central regulatory functions e.g. at the hypothalamic level or as a combination of these possibilities. In this context it should be mentioned that there may also occur inhibition of secretory impulses at various central and/or peripheral levels as e.g on the effector cell or in the autonomic ground plexus (cf III). Re flexes could also be peripheral and local and relay in the inferior mesenteric ganglion (cf. Crowcroft, Holman & Szurszewski 1971) or maybe in the paracervical ganglia.

Uterine secretion is important under various conditions. The possibility that the secretion contains *pheromone(s)* has to be considered. It is however more likely that vulval secretions contain pheromones. Pheromone secretion of the guinea-pig supra-caudal sebaceous glands has been postulated (Parkes 1960) Odorous substances are probably more significant for mammals other than man. Another possible function of the mucus would be as a *lubricant* However secretions from the vagina and the vulval glands probably contribute to a greater extent to this function.

Uterine secretion is probably of great import ance for *spermatozoal transport survival and capacitation*. The migration of spermatozoa from the vagina to the uterine cavity is dependent on the cervical secretion i e. the amount as well as the type of mucus secreted. The migration is facilitated at ovulation and is prevented in late secretory phase (diestrus) The changes in secretion are evoked by female sex hormones, mainly estrogen (Homburger et al 1963 Schmidt Matthiesen 1963 Moghissi 1973a, b, Odeblad 1973 Hamner 1973 Chrétien 1974 1977) Both cervical and corporal secretion probably contributes to the survival of the spermatozoa (Chang 1967 Hamner 1973 Moghissi 1973b, Chrétien 1977) Cervical crypts act as reservoirs of spermatozoa and spermatozoal metabolism is increased i the uterine cavity (Gibbons & Mattner 1971 Moghissi 1971 Chrétien 1977) It is evident that capacitation of the sperma tozoon requires exposure to uterine and/or tubal secretion (Chang 1967 Chang & Hunter 1975)

Further important roles for uterine secretion, mainly the corporeal, have been postulated such as *nourishment of the blastocyst* when it reaches the cavum uteri (Cartier Moricard & Moricard 1960 Shettles & Schmidt Matthiesen 1963 Chang 1967 Beier Petry & Kühnel 1970) and during implantation until a placenta is developed For marsupials uterine secretion could be of utmost importance

Furthermore the secretory products may create an *immunological barrier* against spermatozoa and the fetus which could be considered as a homograft (Bradbury Billington & Kirby 1965 Bradbury et al 1969) Uterine secretion, mainly cervical secretion, is known to contain immunoglobulins and could thereby form a protection against microorganisms (Hulka & Omran 1969 Moghissi 1973b, Chrétien 1977)

Since during a great part of the reproductive cycle neurogenic secretory responses of the guinea-pig endometrium could be elicited, nervous modulation of uterine secretion could be of significance in various situations.

Regarding possible functions of the uterine secretion as a lubricant or as pheromone(s) increased firing in secretomotor fibres may occur at e.g. estrus or during sexual excitement. Decreased neurogenic secretion could take place during e.g. anxiety and fright.

Neuronal discharges during sexual excitement may increase and change uterine mucus in such a way that transport, migration, survival and capacitation of spermatozoa are facilitated. It is also possible that copulation as well as deposition and/or entrance of sperm into different parts of the female genital tract may reflexively influence uterine secretion via secretomotor nerves. On the other hand, during unfavourable conditions neurogenic inhibition of uterine mucus secretion may prevent conception and might participate in regulation of animal population. In women such inhibition could be one possible cause of psychosomatic infertility

Changes in uterine secretion, mainly the corporeal, due to secretomotor impulses elicited by adjustments in central regulation or due to reflexes may also facilitate nutrition of the blastocyst before, during and/or after implantation until placentation has been established Such alterations in uterine mucus secretion, may also facilitate nidation. There is also the possibility that decreased cholinergic stimulation of uterine glandular cells caused by e.g. a peripheral adrenergic suppression of transmittor release may delay the time of maximum apocrine secretion until the blastocyst enters the uterine cavity hence reserving mucus for the crucial events. On the other hand a decreased muscarinic stimulation of mucus cells may inhibit secretion and thereby implantation.

Changes in uterine secretion due to secretomotor discharge may of course be of immunological significance with respect to the events leading to fertilization of the ovum as well as to the events leading to implantation and further development of the conceptus.

In conclusion, since uterine mucus is of great importance to mammalian reproduction, modulation of it caused by varying degrees of muscarinic stimulation of the secretory cells could be of significance for reproduction. As with many other factors influencing fertility the proper role of the secretomotor nerves may vary due to the situation and the species concerned The work presented in this thesis points out that attention should be taken to secretomotor nerves whenever female fertility is considered

V SUMMARY

1 Secretory responses were measured as carbohydrate release from endometria of isolated everted uterine horns from guinea pigs. The presence of acetylcholinesterase positive nerves in the guinea pig endometrium was studied

2. Transmural field stimulation produced an increase in carbohydrate release from uteri brought into artificial secretory phase by a combined treatment with an excess of estrogen and progesterone. The secretory response was abolished by tetrodotoxin and scopolamine but not by phentolamine or sotalol. Acetylcholinesterase positive nerves were found in the endometrium close to glandular cells.

3 The response to field stimulation was reduced after section and degeneration of the hypogastric nerves. It was essentially eliminated after destruction of the paracervical ganglia. Neither previous section of the pelvic nerves nor chronic interruption of the costouterine connexion produced any overt effect on the response. Stimulation of the hypogastric nerve but not stimulation of the pelvic nerve produced a definite increase in uterine carbohydrate secretion.

4 Carbachol but not methoxamine or isoprenaline produced a similar increase in uterine secretion as did field stimulation. The response to carbachol was blocked by scopolamine. Like the response to field stimulation the response to carbachol was of the all or none type and showed little if any gradation

5 Uteri of spayed animals treated with merely estrogen responded to field stimulation and to carbachol but the answers were smaller and required higher concentrations of carbachol than those of uteri from animals treated with both estrogen and progesterone. Uteri from spayed animals treated with merely progesterone did not respond to field stimulation or carbachol.

6. Except during the late diestrus phase, secretory responses to field stimulation and to carbachol could be elicited throughout the estrous cycle. The responses were of a smaller magnitude than those of uteri brought into artificial secretory phase. The sensitivity to carbachol was however the same. At metestrus (day 3—5) in estrous cycle evidence of an inhibitory possibly neurogenic, adrenergic influence on the secretory response to field stimulation was obtained

7 Secretory responses to field stimulation and carbachol were noted at day of implantation in pregnancy During pregnancy responses of increasing magnitude were obtained. In late pregnancy the response was of similar magnitude as that of uteri brought into artificial secretory phase. Secretory responses persisted post partum.

8 It is concluded that the guinea-pig endometrium possesses a cholinergic secretory innervation. The innervation seems to be essentially sympathetic with pre and postganglionic fibres running in the hypogastric nerve. The former fibres relay on short neurons in the paracervical ganglia. The receptors on secretory cells are muscarinic.

9 It is further concluded that neurogenic secretory responses can be elicited during all the important stages of the reproductive cycle of the female guinea-pig. The all or none character of the secretory responses studied could be due to an apocrine type of secretion.

10. It is suggested that neurogenic modulation of uterine mucus secretion could be of importance for fertility This possibility should be considered whenever mammalian fertility is discussed.

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UNIVERSITY OF GÖTEBORG AND KAROLINSKA INSTITUTE, STOCKHOLM, SWEDEN

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ABSTRACT

The vagal control of the pyloric motor function. A physiological and immunohistochemical study in cat and man.

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In acute experiments in anesthetized cats (n = 75) with ligated adrenals the influence on the pyloric sphincter of afferent and efferent electrical stimulation of the cervical vagi was studied with a flow recording technique. Gastric motility was recorded simultaneously with a volume recording technique. *Efferent* stimulation using high threshold parameters (8 V 5 ms, 8 Hz) resulted in a prompt gastric contraction and delayed pyloric contraction (23 ± 17 s). In one third of the cats *biphasic* pyloric motor response consisting of a short period of increased flow preceding a longlasting cessation of the flow was observed. When the transpyloric flow was reduced by splanchnic nerve stimulation or noradrenalin infusion, efferent vagal nerve stimulation induced an *increased flow* supporting the assumption of relaxatory fibres to the pylorus within the vagus as well. Unilateral *afferent* stimulation resulted in decreased flow within 5 ± 3 and a prompt gastric relaxation, which were both abolished at transection of the intact contralateral vagus indicating vago-vagal reflexes.

After atropine (0.2 mg/kg i. b.w.) *efferent* stimulation resulted in gastric relaxation, while the biphasic pyloric motor response was even more pronounced with a long (60 s) latency of the contractile phase. Addition of guanethidine (2 mg/kg i. b.w.) did not affect these responses. After hexamethonium (25 mg i.v. + 50 mg ± 10 mg i.a./h) the stimulation procedure resulted in a gastric relaxation, while the pyloric contraction was blocked. The relaxatory phase required the addition of atropine for blockade indicating separate transmission mechanisms for the two components of the pyloric motor response at such stimulation. Hexamethonium effectively antagonized the pyloric contraction at afferent stimulation.

Immunohistochemical studies revealed rich enkephalinergic innervation of the pylorus and the presence of enkephalin-like material within vagal axons. VIP-like material was demonstrated within the same tissues with a similar distribution. The hypothesis of transmission via non-classical receptors was corroborated in experiments, where the pyloric contraction at vagal *efferent* stimulation was blocked dose-dependently by the opiate receptor antagonist, naloxone i.a. Furthermore micromolar doses i.a. of enkephalins mimicked the vagal motor effects, i.e. pyloric contraction and gastric relaxation, *which were reversed by equimolar doses of naloxone in favour of a pyloric enkephalinergic contrac*tion. Intraarterial injection of nanomolar doses of thus VIP resulted in both a gastric and pyloric relaxation.

Specimens of the human vagus from the thoracic and abdominal levels were investigated with immunohistochemistry. Substance P and enkephalin containing fibres and a low number of VIP fibres were seen at all levels indicating the presence of an axonal transport of these peptides in the human vagus.

Key words. Vagal nerve, electrical stimulation, neural transmission, immunohistochemistry, peptides, motor activity, pylorus, stomach, cat and man.

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The present summary is based on the following publications:

I The vagal control of the feline pyloric sphincter
Edin R., H. Ahlman and J Kewenter
Acta physiol. scand. 1979 107 169–174

II The transmission mechanism of the vagal control of the feline pylorus.
Edin R., H. Ahlman, A. Dahlström and J Kewenter
J Neur Transm. 1980. Accepted.

III Evidence for vagal enkephalinergic neural control of the feline pylorus and stomach.
Edin R. J Lundberg, L. Terenius, A. Dahlström, T Hokfelt J Kewenter and H. Ahlman.
Gastroenterology 1980 In press.

IV On the VIP-ergic innervation of the feline pylorus.
Edin R. J Lundberg, H. Ahlman, A. Dahlström J Fahrenkrug T Hokfelt and J Kewenter.
Acta physiol scand. 1979 197 185–187

V Substance P VIP and enkephalinlike immunoreactivity in the human vagus nerve.
Lundberg, J., T Hökfelt, J Kewenter G Pettersson, H. Ahlman R. Edin, A. Dahlstrom, G Nilsson, L. Terenius, K. Uvnäs-Wallensten and S Said
Gastroenterology 1979 77 468–471

These papers will be referred to in the text by their Roman numerals.

ABBREVIATIONS

ACh	=	Acetylcholine
Mo	=	Morphine
NA	=	Noradrenalin
A	=	Adrenalin
Atr	=	Atropine
Gua	=	Guanethidine
Hex	=	Hexamethonium
Nal	=	Naloxone
VIP	=	Vasoactive intestinal polypeptide
ENK	=	Enkephalin
SOM	=	Somatostatin
SP	=	Substance P
b w	=	body weight
i.a.	=	intraarterial
i.v	=	intravenous

INTRODUCTION

The gastroduodenal junction was named the pylorus (from the Greek pylé-gate ouros-warder-pylorus) since it was believed to be the gateway to the intestine The pylorus has been considered a separate functional entity subject to a special extrinsic innervation and reflex activation (Gaskell, 1916). In accordance with this view the vagus was at first considered to give only motor innervation to the pyloric region (Openchowski, 1889) Later Agostoni et al. (1957) demonstrated that most of the subdiaphragmatical vagal axons were sensory and unmyelineated. Cunningham (1906) pointed out two components of the pylorus: One for closing (m. sphincter) and one for opening (m. dilatator) the lumen.

Anatomically the pylorus is well defined in most species by its conspicuous thick circular muscle layer forming two thirds of the thickness of the wall of the pylorus (Rüdinger 1879 Torgersen, 1942). The sphincter component of the pylorus is formed mainly by the circular muscle layer and probably also by helical fibres, which are longitudinal in the stomach and more circularly arranged around the pyloric orifice (Didio-Andersson, 1968). The dilatator component is composed of longitudinal muscle fibres (Belding & Kernohan, 1953), which are external to and penetrating into the circular muscle layer (Horton, 1928 Muller 1963) The longitudinal muscle fibres form one third of the wall and are intertwined with the circular muscle fibres of the antrum and inserted into the submucosal connective tissue of the gastroduodenal junction. A few superficial longitudinal muscle fibres are continuous with the corresponding duodenal muscle layer

From the literature the conclusion may be drawn that the vagi convey both excitatory and inhibitory motor responses not only to the stomach but also to the pylorus (Katz & Winkler 1902 Cannon, 1906 Thomas & Wheelon 1922 Martinson & Muren 1963) A vagal inhibitory innervation of the pylorus was first suggested by Langley (1898) and later confirmed by May (1904). Subsequent work tended to discredit the idea of a special nervous control of the pylorus and rather established the view of pylorus as the last and most vigorous part of the antro-pyloric pump mainly participating in locally generated peristaltic activity (Edwards & Rowlands, 1968). In 1965 a high pressure zone of the gastroduodenal junction in dogs was demonstrated by Brink et al. (1965). Later this finding was confirmed in man using similar manometric techniques by Fisher & Cohen (1973) who demonstrated an increased intrapyloric pressure after duodenal perfusion with hydrochloric acid, olive oil or amino acids, and a decreased pressure at antral peristalsis. The physiological characteristics of a gastrointestinal sphincter proposed by Cohen et al. (1968) and Fisher & Cohen (1973) were thus fulfilled for the pylorus and expressed as follows

a) A sphincter region which exhibits an intraluminal pressure greater than that of the cavities separated by the sphincter

b) Appropriate stimulation proximal to the sphincter results in a consistent fall in the pressure within the sphincteric zone

c) Appropriate stimulation distal to the sphincter results in a prompt rise in sphincteric pressure

In vitro studies with electrical field stimulation (Anuras et al., 1974) in opossum, cat dog and man suggest an inhibitory innervation of pyloric muscle cells. Efferent vagal nerve stimulation in dogs pretreated with atropine causes an inhibition of spontaneous pyloric motor activity indicating an atropine-resistant inhibitory innervation of the pyloric region (Mir et al., 1979) It has also been shown that the relaxation and "off motor response" in the pylorus in dogs following vagal nerve stimulation are nervemediated, since tetrodotoxin blocks these responses (Telford et al., 1979)

The integrated motor function of the stomach is very complex. It acts as a receptacle for food and as a digestive organ by converting solid food into chyme which is successively emptied into the duodenum. This is an oversimplification since food is emptied both as in liquid and solid form after a meal. The solid particles are emptied by peristalsis of the antral part of the stomach. Liquids or chyme do not depend on peristalsis in order to be emptied but on slow tonic pressure changes in the fundus-corpus part of the stomach (Code & Carlson, 1968 Mroz & Kelly 1977) The antral peristalsis has a speed of 3 cycles per minute in man, and vagal activation increases the amplitude of the peristaltic waves rather than changes their speed (Edwards & Rowlands 1968) There is also a retropulsion of solid particles, which means that food returns from the distal antrum to the fundus-corpus part of the stomach. The pylorus has also been ascribed a role in this process (Carlson et al., 1966)

It was noted early that the gastric emptying of various liquid meals is faster when a high duodenal fistula is allowed to drain the stomach preventing the gastric contents from contact with the duodenal mucosa (Thomas et al 1934) indicating the existence of a "duodenal brake mechanism" In patients after a partial gastrectomy emptying has been shown to be approximately twice as rapid as in controls (Berger 1969) Furthermore the gastric emptying rate was found to be increased in patients where resection was combined with pyloroplasty and vagotomy as well (McGill et al., 1969)

The pyloric sphincter has another important action in preventing bile reflux from the duodenum into the stomach (Fisher & Cohen 1973 Valenzuela, 1976) a mechanism which may be important in the pathogenesis of peptic ulcer as pointed out by DuPlessis (1965) Because of the late complications of gastric resections such as the dumping syndrome and the development of carcinoma in the resected stomach, most surgeons favour highly selective vagotomies rather than gastric resections in order to save the pyloric function. The motor control of the pylorus may be exerted by several factors such as luminal stimulants, gastrointestinal hormones and the intrinsic and extrinsic nervous supply of the gastroduodenal region The aim of the present study was to evaluate the role of the extrinsic vagal control of the pylorus in an experimental model in cats.

AIM OF THE INVESTIGATION

The gastroduodenal junction is protected by a sphincter which fulfills the described characteristics of such a sphincter (I) Only a few studies have dealt with the extrinsic nervous control of the pyloric sphincter and the results are conflicting. Furthermore in addition to the classical transmitters NA and ACh, there is now evidence that other newly discovered transmitter substances, such as small peptides are to be found in the vagus nerve (Lundberg et al 1978 a b) These substances may serve as transmitters (cf Otsuka & Takahashi 1977 Fahrenkrug et al., 1979) involved in a vagal control of the pyloric sphincter The aim of the present investigation was therefore

1 To study *in vivo* the effects of vagal nerve stimulation on an externally applied flow of saline through the feline pyloric sphincter by means of a flow recording method. Furthermore gastric motility was studied simultaneously in order to investigate whether the vagal nerves might exert specific effects on the pyloric region separate from those on the stomach. To study the effects of cholinergic, adrenergic and ganglionic blocking agents on the pyloric motor responses obtained at efferent and afferent electrical stimulation.

2 To study by immunohistochemistry the possible occurrence of possible neurotransmitters within neurons of the gastric or pyloric wall of the cat

3 To study the physiological effects of possible neurotransmitters, such as peptides localized to intramural neurons on pyloric and gastric motility *in vivo* in cats when administered locally via the splenic artery

4 To demonstrate the existence of possible neurotransmitters, other than ACh and NA, in the vagal nerve in man by immunohistochemistry

MATERIAL AND METHODS

Physiological experiments

Operative procedure

Experiments were performed on 75 adult cats of both sexes. The number of animals used in the various series is given in each paper A few cats were used for experiments in several different series. The animals were fasted for 24 hours but had full access to water Anesthesia was initiated with ether and continued with chloralose (50 mg/kg b w) slowly injected through one of the femoral veins. A tracheal cannula was inserted to maintain a free airway and all animals breathed spontaneously except in a few experiments in which a ventilator was used. The animals were placed on an operating table with a thermostatically regulated heating pad in order to maintain the body temperature at about + 38°C A femoral artery was cannulated and connected to a pressure transducer (Statham p 23 DC) for continuous recording of arterial blood pressure.

The abdomen was opened with a midline incision. Since catecholamine secretion from

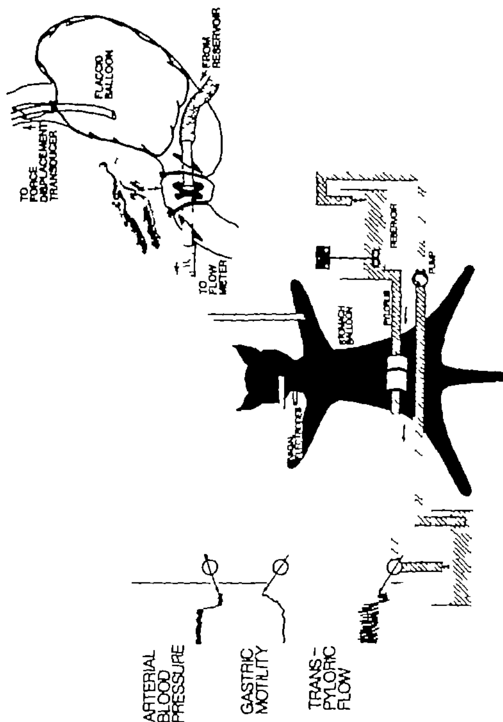


Fig. 1 Experimental model to study gastric and pyloric motility simultaneously in the anesthetized cat (for details, see text). *Inset* Detail of the set-up to study the transpyloric Glass tubes separated only by the sphincter itself (see histology section).

the adrenal medulla is known to be a potent intestinal inhibitor (Kock, 1959) the vessels of these glands were ligated at the beginning of all experiments. The duration of each experiment was less than 4 hours and corticosteroid substitution was therefore not given.

Recording of transpyloric flow

A non-compressible plastic tube (diameter 8 mm) was inserted through a small longitudinal incision along the greater curvature of the anterior wall of the lower corpus region of the stomach and secured with a ligature immediately proximal to the pylorus. Consequently antral motor activity could not influence the transsphincteric flow A similar plastic tube was inserted through a longitudinal incision in the descending part of the duodenum and brought up to the pylorus just opposite the proximal tube In this position the distal tube was secured with a ligature around the duodenum (Fig. 1)

A reservoir containing physiological saline and thermostatically regulated to 38°C, was connected to the proximal tube The distal tube was connected to a photoelectric flow meter which recorded volume changes of the transpyloric flow between 20 – 1000 ml/min The saline from the flowmeter was collected and constantly pumped back to the perfusion reservoir Since the fluid surface of this reservoir was large (370 cm^2), the inflow pressure through the sphincter could be kept almost constant recirculating the saline The pressure gradient between the two reservoirs and the pylorus could be varied by adjusting the level in the reservoirs. The average height between the reservoirs was 50 cm. To initiate the inflow of saline through the pylorus a pressure of 20 cm H_2O was induced and then reduced to about 10 cm H_2O (Fig. 1)

Recording of gastric tone

A deflated plastic balloon of about 1000 ml volume was introduced orally into the corpus-fundus part of the stomach of the anaesthetized animal. The balloon was connected by a rubber tube to a water tank mounted on a weight recorder (Grass Force Displacement Transducer FT 10 C) and connected to an amplifier. The water level in the tank could be varied by changing the level of the weight transducer By opening the system with the tank at different water levels in relation to the stomach, water was allowed to fill the gastric balloon In most experiments such a hydrostatic pressure gradient was accomplished at 5 cm H_2O Blood pressure intragastric tone and transpyloric flow were simultaneously recorded by means of a Grass polygraph (Grass S4 E) (Fig. 1)

Nerve operations and stimulation procedures

The cervical vagal nerves were isolated through the tracheostomy incision from the sympathetic trunk and divided uni- or bilaterally The proximal and distal cut ends of the vagus were inserted separately into insulated ring electrodes, which were placed deep into the wound before nerve stimulation. In 16 cats, given *efferent* vagal nerve stimulation, the splanchnic nerves were left intact in 5 animals and in 11 animals divided bilaterally beneath the diaphragm at the preganglionic level before stimulation.

In animals where the unilateral *afferent* vagal nerve was stimulated the splanchnic nerves were left intact in 7 and divided bilaterally in 11 cats. In 4 animals both the splanchnic and contralateral vagal nerves were divided during the experiment. All nerve stimulations were performed using square wave pulses from a Grass stimulator (S4 E) over 1 – 10 min. The stimulation parameters (8 – 15 V 0.2 – 5 ms 8 – 12 Hz) were divided into 2 groups activating *high* (5 ms) and *low* threshold (0.2 ms) fibres selectively according to Martinson (1965)

Drug infusion

A fine plastic catheter (diameter 0.5 mm) was introduced via the splenic artery in a retrograde direction and the tip of the catheter placed at the junction with the celiac artery The infusion of ink through this catheter after the experiments showed a distribution to the stomach, pylorus, and proximal part of the duodenum. The substances were dissolved in saline at body temperature before the infusion.

Drugs

Atr (Atropine sulfate Merck 0 1 – 0.2 mg/kg) and Gua (Guanethidine sulfate CIBA 2 mg/kg) were given i.v Hex (Hexamethonium chloride Fluka AG) was given as a bolus dose i.v (25 mg/kg) followed by an i.a. infusion (50 ± 10 mg/kg b w hr) Mo (Morphine chloride ACO Sweden, 0 1 – 1 mg; 0.3 – 3 1 µmol) (D – Ala_2 met^5) – enkephalinamide (Bachem, Bubendorf Switzerland, 0.2 – 1 mg, 0.3 – 1.32 µmol) [met^5]- and [leu^5] enkephalin (Bachem Switzerland) (0.05 – 0.01 mg; 0.08 – 0 16 µmol) ACh (Acetylcholine chloride Roche 0.05 – 0 1 mg, 0.3 – 0 6 µmol) VIP (generously supplied by Professor Viktor Mutt Dept of Biochemistry Karolinska Institute Stockholm Sweden, 0 1 – 4 µg; 0.03 – 1.3 nmol) were given as bolus doses i.a. Nal (Naloxone chloride Endo) was given at an infusion rate of 0 1 – 0 4 mg/kg b w min at a total dose of 2.6 – 6 mg (6.9 – 16 6 µmol) or as a bolus dose of 0 1 – 0 4 mg (0.3 – 1.2 µmol).

Immunohistochemistry

Preparation and characteristics of antisera

All antisera were raised in rabbits using SP VIP and met ENK conjugated to bovine serum albumin. Characterization of these antisera revealed that the SP antiserum (K25) crossreacts to a negligible extent (< 0.1 %) with ENK, VIP SOM glucagon, bradykinin, and eledoisin (Nilsson et al., 1975). The VIP antiserum (5603–5) shows negligible crossreactivity (< 0 1 %) with secretin, glucagon cholecystokinin-pancreozymin pancreatic polypeptide, SP SOM neurotensin, alfaendorphin leu and met ENK bradykinin and angiotensin (Fahrenkrug et al. 1977). Finally the met ENK antiserum (336) shows negligible crossreactivity (< 0 1 %) with the large molecular weight endorphins SP VIP and SOM but crossreacts to about 25 % with leu-ENK (Schultzberg et al., 1978) Characterization of these antisera was carried out with high dilutions of the antisera in radioimmu-

noassay models and later also in experiments with more concentrated dilutions, where each antiserum was preabsorbed with the other two peptides (100 μg of each peptide in a final antiserum dilution 1 100) without changes in the immunofluorescence staining. It cannot be excluded however that the antisera may bind to unknown peptides with similar amino acid sequences. It must also be pointed that the peptides used for antibody production were originally isolated and sequenced from porcine or bovine tissues, and that the exact structure of the corresponding human or feline peptides is unknown. It therefore seems appropriate to use terms such as SP-like ENK-like and VIP-like immunoreactivity or immunoreactive material.

Immunohistochemical procedure

Six adult cats of both sexes were used. Three of these were treated with local applications of vinblastine (Sigma) (Dahlström, 1971) on the serosal surface of the corpus, antral and pyloric regions of the stomach. Twentyfour hours later the stomachs were perfused with 2 000 ml of ice cold 4 % phosphate-buffered formaldehyde. The vinblastine treatment is known to raise peptide levels of the nerve cell bodies because of interrupted axonal transport (Dahlstrom, 1971 Lundberg et al. 1978). The three parts of the stomach were dissected out and immersion fixed for .. hours in the same fixative and then rinsed in 5 % sucrose in phosphate buffer at + 4°C overnight.

The specimens were then processed for the indirect immunofluorescence technique according to Coons (1958). Briefly 10 μm thick consecutive sections were incubated with antisera to met-ENK, VIP or SP (preabsorbed with BSA 50 mg/ml) when diluted 1 10, in a final dilution of 1 100 – 200 (containing 0.3 % Triton X 100) for 24 hours at +4 C. The sections were then rinsed in phosphate-buffered saline (PBS) and incubated with FITC (fluoresceinisothiocyanate)-conjugated sheep antirabbit antibodies (SBL, Stockholm Sweden) diluted 1 4 for 30 min at 37°C. The sections were rinsed again in PBS then mounted in PBS-glycerine (1 3) and examined in a Zeiss fluorescence microscope equipped with an oil dark field condensor and a Schott BG–12 or alternatively a KP 500 excitation filter and a Zeiss 50 or LP 250 stop filter Adjacent control sections were incubated with the antisera preabsorbed for 24 hours with an excess of respective peptide i.e. 100 μg met-ENK/ml antiserum diluted 1 160, 50 μg VIP or SP/ml antiserum diluted 1 100

Collection of vagal nerves

Previous studies on neuropeptides in the axons of other species have indicated the necessity of a nerve crush (Lubinska, 1959) to raise the peptide levels to a level detectable with immunohistochemistry (Lundberg et al., 1979).

In 8 patients undergoing various kinds of thoracic or abdominal surgery 2 right thoracic main trunks, ? anterior subdiaphragmatic trunks and 4 anterior nerves of Latarjet were collected. 30 min to 4 h before dissection the nerves were crush-operated with a silk suture compressing the nerve against a glass or metal rod for 5 sec and then released. The

segment 10 mm above and 5 mm below the crushed nerve was dissected and immersion fixed in ice-cold 4 % phosphate-buffered paraformaldehyde for 8 hours and rinsed in phosphate buffer containing 5 % sucrose over night. The nerves were then processed for the indirect immunofluorescence technique as described in (V).

METHODOLOGICAL CONSIDERATIONS

The vast majority of previous *in vivo* studies of the pylorus region have been performed by means of pressure recordings or recordings of the electrical activity (Thomas & Wheelon, 1922, Brink et al 1965 Fisher & Cohen 1973 Mroz & Kelly 1977). Pressure recording systems have some disadvantages when gastrointestinal sphincters are investigated It may be difficult to keep the pressure receptor within the sphincter region, which may also be difficult to exactly identify Furthermore the recording device might interfere with sphincter motor activity Recordings of electrical activity or contractility recorded by extramural strain gauge force transducers do not allow the study of overall motor function in such regions of the gastrointestinal tract. This may explain some of the differences between previous studies of the pyloric sphincter In order to avoid some of these disadvantages and to be able to study the overall motor function of the pyloric region a flow recording method was used Thus, the interference from a balloon or catheter within the sphincter region was avoided and even small variations in sphincter tone could be studied. However when a perfusion method is used several factors have to be taken into consideration. The perfusion pressure has to be kept at a constant level using a reservoir with a large area and a recirculation pump The perfusion and drainage tube must have a capacity which is larger than the maximal flow through the sphincter and the tubes have to be placed close to the sphincter in order to avoid secondary effects from the adjacent antrum and duodenum. Since saline was used as perfusion fluid, interference due to viscosity could be excluded The temperature of the perfusion fluid has been shown to be of great importance (Pahlin 1975) Therefore, the temperature of the perfusion fluid was kept constant at 38 C

As has been pointed out by Cruveilhier (1845) Wertheimer (1891) and Chvalero (1979) the stomach has a segmental innervation and the gastric vagal nerves have a fairly constant course The pyloric area is mainly innervated by the pyloric branches from the hepatoduodenal nerve of Latarjet and also by the anastomosing branch from the anterior nerve of Latarjet (Chvaero 1979). Both nerves innervate the pylorus from the hepatoduodenal ligament cranial to the ligatures. Great care was taken to exclude the mesentery of the stomach and the hepatoduodenal ligament with its vagal nerve supply to the pylorus from nerve lesions imposed at dissection.

In the experiments with i.a. drug infusions a catheter was introduced via the splenic artery in a retrograde direction with the tip of the catheter placed at the junction with the celiac artery On terminating the experiments an ink infusion through this catheter showed a distribution to the relevant region that is the antrum, pylorus and proximal part of the duodenum in all animals.

RESULTS

Physiological experiments on vagal neurotransmission by classical receptors

Efferent vagal nerve stimulation (I)

Uni- and bilateral efferent cervical vagal nerve stimulation with activation of *high* threshold fibres elicited a reduction or cessation of the transpyloric flow after about 20 sec, whereas a rapid (within 4 – 5 s) contraction was obtained in the stomach.

In 10 of 29 animals efferent vagal stimulation of the *high* threshold fibres resulted in a small but clear immediate initial increase of the transpyloric flow followed by a reduction of the flow. There was no difference in the pyloric or gastric response whether the splanchnic nerves were cut or left intact (Fig. 2 a).

Unilateral vagal stimulation activating the *low* threshold set of fibres, reduced the transsphincteric flow within 20 seconds in 2 of 5 cats. Bilateral stimulation activating the *low* threshold fibres, reduced the transsphincteric flow in only 1 of 4 animals.

Efferent vagal nerve stimulation during concomitant splanchnic nerve stimulation or NA infusion (I)

In order to investigate the influence of sympathetic tone on the pyloric motor response, the vagal nerves were stimulated when the sphincter tone was increased by a concomitant splanchnic nerve stimulation or local *i.a.* infusion of NA. The effect of vagal stimulation during simultaneous splanchnic nerve stimulation was studied in 5 experiments. Splanchnic nerve stimulation with supramaximal currents at 8 Hz elicited a contraction of the pylorus. Bilateral efferent vagal stimulation with activation of the *high* threshold fibres caused an *increased* transsphincteric flow in all animals.

In 2 animals the effect of vagal stimulation before and during NA infusion (0.01 – 0.07 mg/kg b.w./min i.a.) was studied. The infusion caused in these experiments a contraction of the sphincter. Efferent vagal stimulation during concomitant NA infusion caused an *increase* of the reduced flow in both animals studied.

Efferent vagal nerve stimulation after pretreatment with atropine, guanethidine and hexamethonium (II)

When 29 animals were pretreated with Atr (0.2 mg/kg i.v.) the nerve stimulation with *high* threshold parameters resulted in a gastric relaxation in all animals, whereas the pyloric motor response had a pronounced *biphasic* appearance in 24 animals consisting of an initial relaxation followed by a contraction some 60 sec later (Fig. 2 b).

In 5 animals the pyloric motor response changed into a pure *monophasic* relaxation. The vagally induced relaxation of the stomach and the pylorus was prompt, whereas the onset of the pyloric contraction had a significantly ($p < 0.005$) longer latency after Atr than the pyloric contraction in the untreated animal.

In 9 animals Gua was combined (2 mg/kg i.v.) with Atr. In all of these animals the gastric relaxation and biphasic pyloric motor responses recorded at nerve stimulation were similar to those recorded from animals given Atr only.

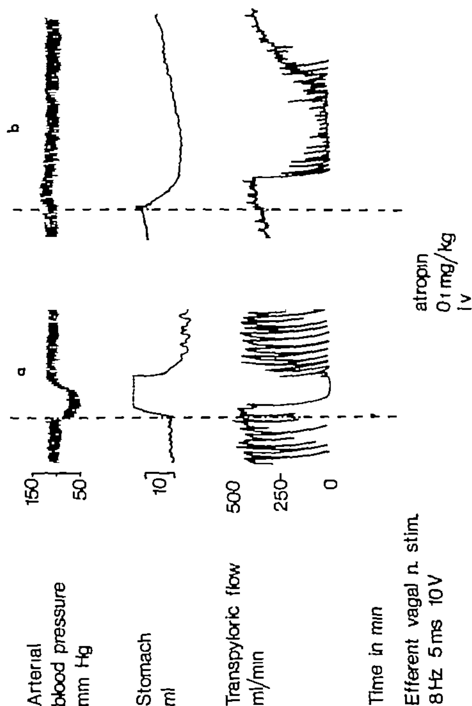


Fig. 2. The effect of efferent vagal nerve stimulation of the high threshold fibres on i.a. blood pressure, gastric and pyloric motility before and after the administration of atropine (0.1 mg/kg i.v.). Nerve stimulation in this animal resulted in a prompt gastric contraction and a late cessation of the transpyloric flow. However after atropine gastric relaxation and a pronounced biphasic pyloric motor response was obtained with an initial increase and a *delayed* cessation of the flow. Note also the absence of blood pressure reaction after the drug pretreatment.

In 12 cats nerve stimulation was carried out after Hex (25 mg/kg i.v. followed by 50 ± 10 mg/kg b.w./hr i.a.). Gastric motility was recorded in 10 of these animals. In 9 cats stimulation resulted in a gastric relaxation. In 11 cats there was a vagally induced pyloric relaxation, and in one cat there was both a gastric contraction and pyloric contraction.

When Atr was added to Hex in these 12 cats (or Hex was added to Atr in 4 other animals) the gastric motor response on nerve stimulation was completely blocked in all 16 animals. The pyloric relaxation was blocked in 14 animals and persisted in 2 animals.

Afferent vagal nerve stimulation (I)

Afferent stimulation of the cervical vagal nerve with the contralateral nerve intact resulted in a reduction of the transpyloric flow and a concomitant gastric relaxation when the *high* threshold fibres were activated. Activation of the *low* threshold fibres resulted in a pyloric contraction in a few animals (Fig. 3). Reduction of the transphincteric flow on afferent nerve stimulation followed after 5 ± 3 s, whereas gastric relaxation was always prompt.

In 4 animals with a preganglionic denervation of the splanchnic nerves the cut proximal end of the vagus was afferently stimulated before and after division of the contralateral vagus. Vagal stimulation elicited a pyloric contraction and gastric relaxation in all 4 animals. These responses disappeared completely after transection of the contralateral vagal nerve. Efferent stimulation of either vagal nerve again induced a contraction of the pylorus.

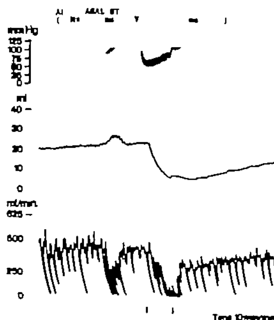


Fig. 3. The effect of *afferent* vagal nerve stimulation using *low* and then *high* threshold parameters on i.a. blood pressure, gastric and pyloric motility. Activation with either *low* or *high* threshold parameters elicited pyloric contraction, only the *high* threshold parameters elicited gastric relaxation.

Afferent vagal nerve stimulation after pretreatment with atropine, guanethidine and hexamethonium (II)

Afferent vagal nerve stimulation was given after pretreatment with Atr in 14 cats. The vagally induced contraction was blocked in 3 animals, and remained unaffected in 11 animals. In 5 animals Gua was added to the Atr pretreatment, a procedure which did *not* block the pyloric contraction in any of the animals.

In 10 animals nerve stimulation was carried out after Hex pretreatment In 8 of these 10 animals the pyloric contraction was completely blocked, and in 2 animals it persisted.

Immunohistochemistry of peptides in the vagus and the pylorus

Immunohistochemistry of the pylorus and stomach (III)

The two neuropeptides, ENK and VIP were both localized to numerous nerve fibres in the circular smooth muscle layer and the myenteric plexus of the feline pylorus. After pretreatment with vinblastine ENK and VIP immunoreactive nerve cell bodies could be demonstrated in the myenteric plexus of the pylorus. The most striking difference between the pylorus and the stomach was the presence of a dense network of nerve fibres with ENK and VIP-like material running in parallel with the smooth muscle fibres of the circular muscle layer of the pyloric sphincter but only a few fibres were demonstrated within the same layer of the stomach. In vinblastine-treated animals the number of visible ENK and VIP nerve cell bodies of the myenteric plexus was greatly increased, especially in the pyloric region. The number of visible nerve cell bodies was much lower in the antrum – corpus. VIP immunoreactive nerve cell bodies were also observed in high numbers in the submucous plexus together with a dense network of VIP immunoreactive nerve fibres in the mucosal lamina propria of both the pylorus and the stomach. A dense network of ENK positive nerve terminals was also observed in the myenteric plexa of all the gastric regions investigated (see Table I).

Substance P VIP and ENK-like immunoreactivity in the human vagus nerve (V)

Immunoreactive material of the three peptides SP VIP and ENK were found in the axons of the vagal nerves at all levels studied as indicated by the appearance of fluorescent nerve fibres proximal to the ligation. The relative proportions of the peptides in the three branches investigated that is the thoracic main trunk, the anterior subdiaphragmatic trunk, and the anterior nerve of Latarjet were rather similar very high numbers of SP-fibres, somewhat fewer ENK fibres and a few VIP axons in each nerve segment. The accumulation of immunoreactive material above the ligation was seen after 1 hour and increased with time indicating a rapid anterograde axonal transport of the peptides. Thin ENK axons could even be demonstrated far away from the crush on both sides within the nerve of Latarjet Control sections were negative as described in (V).

Table 1 Localization and estimation of the abundance of VIP and ENK-immunoreactive material within nerve fibers and nerve cell bodies in the gastric and pyloric wall of the cat.

	PYLORUS		STOMACH	
	ENK	VIP	ENK	VIP
LAMINA PROPRIA MUCOSAE	–	nerves ++	–	nerves ++
SUBMUCOUS PLEXUS	–	ganglion cells ++	–	ganglion cells ++
CIRCULAR MUSCLE LAYER	nerves ++	nerves ++	nerves (+)	nerves +
MYENTERIC PLEXUS	nerves ++ ganglion cells ++	nerves ++ ganglion cells + (+)	nerves ++ ganglion cells (+)	nerves + ganglion cells (+)
LONGITUDINAL MUSCLE LAYER	nerves (+)	nerves (+)	nerves (+)	nerves (+)

nerves = axons and nerve terminals

(+) few
\+ moderate number or medium
\+ + high number or dense

Physiological experiments on vagal neurotransmission by non-classical receptors

Vagal nerve stimulation before and after naloxone and/or atropine (III)

During infusion of Nal there was an increasing delay of the pyloric contraction whereas the gastric contraction remained unchanged at efferent vagal nerve stimulation using *high* threshold parameters A final blockade of the pyloric response was obtained in all cats examined (6/6) The vagally mediated cholinergic gastric contraction seemed to be unaffected during i.a. infusion of Nal as indicated by the response to ACh injection. When Atr was given to the animals, *high* threshold vagal stimulation still elicited a pyloric contraction whereas the gastric response had changed into a relaxation. During Nal infusion an increasing blockade of the pyloric contraction at vagal nerve stimulation was seen in all 9 cats. A total blockade was observed in 6/9 cats at a total dose of 2.5 mg (6 9 μmol) of the drug. In the remaining 3 animals, there was a dose dependent increasing delay of the pyloric motor response and the contraction was completely blocked at a total dose of 6 mg (16.6 μmol). The Atr resistant gastric relaxation was also affected but to a lesser extent (Fig. 4).



Fig. 4. Recordings f i.a. blood pressure, gastric motility and transpyloric flow in an atropine pretreated (0.1 mg/kg i.) animal during electrical vagal nerve stimulation in efferent direction, using *high* threshold parameters and i.a. infusions f naloxone as indicated in the figure (from III with permission of Gastroenterology).

Intraarterial injections of morphine met and leu-enkephalin and naloxone (III)

The i.a. injection of Mo (8 cats) caused a delayed cessation (within 1 – 3 min) of the transpyloric flow (duration 4 – 5 min) in all cats and induced a slow increase in the gastric tone which lasted for 20 – 30 min in 7 of 8 cats induced with a dose of 0.5 mg (1.5 μmol) or more The lowest tested dose 0 1 mg (0.3 μmol) resulted in a 50 – 70 % reduction of the transpylorie flow (2 cats) which lasted for a few seconds only The administration of equimolar bolus doses of Nal blocked the Mo effects on both the pylorus and the stomach. The infusion of met or leu ENK (0.08 – 0 16 μmol) (5 and 4 cats respectively) elicited an immediate contraction of the pylorus of shorter duration (up to 3 min) than that obtained with Mo This effect on the transpyloric flow was almost abolished by pretreatment with Nal infusions. A prompt gastric relaxation (in contrast to the slow contraction obtained with Mo) was obtained which was also blocked by Nal. Pretreatment with Atr or Hex did not affect the responses elicited by i.a. injection of ENK. The specificity of the Nal blockade was tested by the injection of ACh in 5 cats. ACh induced a cessated transpyloric flow and a gastric contraction before and after the administration of Nal in all animals (Fig. 5)

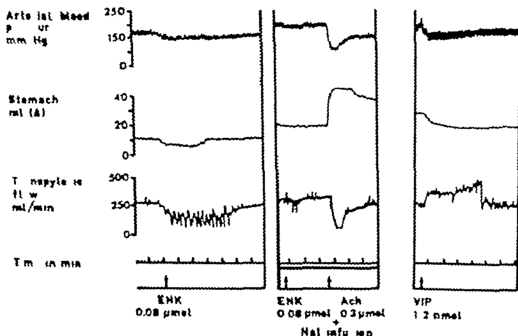


Fig. 5 Recordings of i.a. blood pressure gastric motility and transpyloric flow in the cat during i.a. bolu injections of ENK (0.08 μmol) and VIP (1 2 nmol) respectively ENK caused a pyloric contraction and gastric relaxation, both effectively blocked by naloxon inf sion (total dose 6 9 – 16.4 μmol). The cholinergic receptors seemed intact. VIP caused both a pyloric and gastric relaxation.

Intraarterial injections of VIP (IV)

The intraarterial injection of VIP in 7 cats induced a prompt gastric relaxation and an immediate increase in the transpyloric flow at all doses given (0.03 – 1.3 nmol) but only in 50 % of the experiments with the lowest dose (0.03 nmol). The VIP effects could not be blocked with Atr or Hex at the doses previously used (II) (Fig. 5).

DISCUSSION

Physiological experiments on vagal neurotransmission by classical receptors (I II)

In the present series of experiments *efferent* cervical vagal nerve stimulation with physiological frequencies and supramaximal currents caused a monophasic contraction of the pylorus with a latency of 23 ± 17 s in two thirds of the animals. In the remaining animals, an initial relaxation of the pylorus with an increased transsphincteric flow with a latency of 5 ± 3 s followed by a contractile phase of the sphincter was recorded. This may indicate the presence of both excitatory and inhibitory vagal nerve fibres to the pylorus. To further investigate the vagal control of the pyloric sphincter region the vagal nerves were stimulated during a concomitant increase in sphincter tone since the type of intestinal response to vagal stimulation is considered to be determined by the tonus (Garry 1957 van Harn, 1963 Thus low tonus of the intestine may favour an excitatory response and high tonus an inhibitory response When pyloric tonus was increased with NA infusion or splanchnic nerve stimulation vagal activation caused an increased flow i.e. an inhibitory response in both these series of experiments. This supports the assumption that the vagal nerves convey both excitatory and inhibitory fibres, not only to the stomach but to the pylorus as well as earlier suggested by Langley (1898) Anuras et al. (1974) Martinson (1965 a, b) Mir et al. 1979).

Afferent cervical vagal stimulation with physiological frequencies and supramaximal currents with the contralateral vagal nerve left intact resulted in a contraction of the pylorus within 5 sec, as well as prompt gastric relaxation. Both responses disappeared when the contralateral cervical vagal nerve was cut indicating the presence of a vago-vagal excitatory reflex activation of the pylorus as well as a similar inhibition of the stomach.

It has previously been shown that vagal nerves contain at least two groups of efferent fibres which control gastric motor activity (Martinson and Muren, 1963) The excitatory fibres were selectively activated when the *low* threshold parameters were used If the duration of the stimulatory pulses was increased to supramaximal currents, *high* threshold vagal relaxatory fibres were activated as well. Activation of the *low* threshold fibres induced an inconsistent excitatory motor response of the pylorus in the present series of experiments, but relaxatory responses were not seen. Activation of the *high* threshold fibres caused pyloric motor responses as described above

In order to study the transmission mechanisms involved in the vagally induced excita-

tory and inhibitory pyloric motor responses *high* threshold parameters were used in both efferent and afferent stimulations after the administration of classical cholinergic adrenergic and ganglionic blocking agents.

Efferent vagal stimulation after the administration of *atropine* resulted in a gastric relaxation in all animals thus confirming previous findings (Martinson et al., 1965 a) The pyloric motor response was biphasic in the majority of the animals, an immediate relaxation followed by a delayed contraction with a significantly longer latency than seen before Atr was given. In a few animals a monophasic relaxation of the pylorus was obtained. Motor responses on *afferent* vagal nerve stimulation after Atr were unaffected in the vast majority of the experiments. However in a few animals a blockade of the pyloric contraction was seen. Atr in the doses given in the present series of experiments (e.g. 0 1 – 0.2 mg/kg) is known to completely block the vagally induced excitatory motor effects on both the stomach, the small intestine the ileo-cecal sphincter and the colon (Martinson 1965 Kewenter 1965 Hultén, 1969 Pahlin, 1975). Furthermore the low dose of Atr used in the present series of experiments is considered to have a specific anti cholinergic action on muscarinic receptors (Innes & Nicherson, 1975). However such a blockade did not effectively prevent a pyloric contraction except in a few animals. An additional effect of atropine cannot therefore be completely excluded.

When *guanethidine* was given after Atr the gastric as well as the pyloric motor responses at *efferent* and *afferent* vagal stimulation were similar before and after administration of Gua. Thus, an adrenergic transmission mechanism can be excluded *Hexamethonium* blocked the gastric as well as pyloric contraction in all but one animal on *efferent* stimulation and in all but two cats on *afferent* stimulation, indicating a ganglionic transmission (Volle & Koelle, 1975) However the relaxatory motor responses in both the stomach and the pylorus remained unchanged.

When a combination of Atr and Hex was given, the gastric relaxation was blocked in all animals and the pyloric relaxation in 14/16 animals on efferent cervical vagal nerve stimulation. The total blockade of the gastric relaxation in these experiments is in contrast to the partial blockade described by Martinson et al. (1965 a). However it should be noted that the dose of Hex used here was different and that a constant i.a. infusion of the drug was given throughout the experiments.

In conclusion. Hex alone was effective in blocking the vagally elicited excitatory motor response of both the pylorus and the stomach indicating a ganglionic transmission step. Furthermore, the gastric excitatory responses seem to be mediated by cholinergic transmission, whereas the pyloric excitatory responses do not seem to be mediated by classic al muscarinic receptors. However the non-cholinergic, non-adrenergic relaxatory responses of the pylorus and the stomach required the addition of Atr to Hex to be blocked (Fig. 6).

The results obtained after the administration of cholinergic and adrenergic ganglionic blocking agents on the vagally induced pyloric motor response suggest one or more of the following possibilities of neural transmission. Thus, both nicotinic and muscarinic receptors seem to be involved in the synaptic transmission either at the ganglionic level or the effector cell level, or both, since a combination of Atr and Hex had to be given to block

the relaxatory pyloric motor response. A ganglionic transmission has previously been suggested in the vagal inhibitory control of the lower oesophageal sphincter (Goyal & Rattan 1975) and also for the non-cholinergic non-adrenergic receptive relaxation of the stomach (Martinson, 1965 a, b). Since some of the vagal nerve fibres are postganglionic (Schofield et al. 1968) the occasional persistent vagally induced contractions after Hex on efferent stimulation might also be explained by the activation of such fibres. An antidromic stimulation of *afferent* sensory vagal fibres cannot be completely excluded since such an activation may cause a release of products that relax sphincter muscles (Burnstock, 1972).

An interesting finding in the present experiments was that the pyloric contraction elicited by *efferent* vagal nerve stimulation was seen after a delay of about 20 sec. This is in contrast to the almost immediate contraction seen in the stomach, small intestine, ileocecal valve and colon induced by efferent vagal stimulation (Martinson 1965 a, b; Kewenter 1965; Hultén, 1969; Pahlin 1975). This rather long latency may indicate the activation of composite neurohumoral mechanisms such as the release of gastrointestinal hormones, or simply reflect that the innervation of sphincter regions is more complex than that of the gut itself (Goyal & Rattan 1975). A procedure such as *efferent* electrical stimulation of the vagi activates not only cholinergic and adrenergic fibres, but probably also peptidergic fibres (Uvnäs-Wallensten, 1978; Lundberg et al. 1978 a, b) present in this nerve. Furthermore it is known that monoamines (Ahlman, 1976) as well as peptidergic hormones (Uvnäs-Wallensten et al. 1976) with potent actions on gut motility are released by such stimulations. The delayed pyloric contraction on vagal *efferent* stimulation may therefore be due to a release of such substances or both into the gut lumen and/or into the blood stream. However, it has been shown that the relaxation as well as the "off motor response" of the canine pylorus following vagal stimulation seem to be nerve mediated, since tetrodotoxin blocked these responses (Telford et al. 1979).

In order to find what possible neurotransmitters were responsible for the non-cholinergic non adrenergic motor effects found in the present studies the vagus nerve, the gastroduodenal junction and the stomach were investigated using immunohistochemical techniques for the presence of several neuropeptides.

Immunohistochemistry of peptides in the vagus and the pylorus of the cat (III)

Immunohistochemical studies in the cat revealed that the pyloric region had much denser networks of nerve fibres with ENK-immunoreactive material in the circular muscle layer than the stomach. In contrast to a previous study (Linnoila et al., 1978) ENK-positive nerve cell bodies were found in the myenteric plexuses with a much higher concentration in the pyloric region than in the antrum-corpus part of the stomach. In the myenteric plexus dense networks of ENK-positive nerve-terminallike structures were observed at all levels of the stomach, in agreement with previous studies (Elde et al. 1976; Polak et al., 1977).

The immunohistochemical studies demonstrated numerous VIP immunoreactive cell bodies in both intramural plexuses of the pylorus, with the highest number in the sub mucous plexus. VIP-immunoreactive nerve fibres were seen in the myenteric plexus, circular muscular layer and lamina propria mucosae of the pylorus.

The number of VIP immunoreactive cell bodies in the myenteric plexus was less in the stomach than in the pylorus, but approximately the same amount were found in the submucous plexuses of the pylorus and stomach. The circular muscular layer of the antrum-corpus contained a medium dense network of VIP-immunoreactive nerve fibres while in the lamina propria a dense network was seen and only scattered nerve fibres in the longi tudinal muscle layer.

VIP-containing nerve fibres have previously been demonstrated in other sphincter regions than the pylorus, such as the lower oesophageal sphincter and the sphincter of Od di (Alumets et al., 1978), gastrointestinal and genito-urinary tract (Alumets et al., 1979 Larsson et al. 1976) The numerous VIP-immunoreactive fibres of the lamina propria both in the stomach and the pylorus may be of importance for secretion and mucosal blood flow as suggested by Fahrenkrug et al.(1978 b) and Eklund et al. (1979).

Physiological experiments on vagal neurotransmission by non-classical receptors (III IV)

Since immunohistochemical studies (III) revealed a rich ENK-ergic and VIP-ergic innerv ation of the pyloric region it was of interest to experimentally study if ENK and VIP are involved in the vagal neurotransmission to the pylorus.

When the cervical vagal nerves were stimulated in an *efferent* direction with supramaxi mal currents and physiological frequencies during i.a. infusion with the opiate receptor antagonist Nal the pyloric contraction was inhibited in a dose dependent manner These experiments were repeated after the administration of Atr to investigate the Nal-effect on gastric relaxation. It was then found that the vagally induced gastric relaxation was also influenced by Nal-infusion, but to a lesser extent than the pyloric contraction, and the gastric relaxation was never completely blocked. This suggests that opioid receptors are involved in these vagally mediated motor responses, although the receptors in the pylorus and stomach may be slightly different. In order to further investigate this hypothesis the natural agonists of opioid receptors, enkephalines (leu- and met-ENK), were injected i.a. to the pyloric and the lower stomach regions since if an ENK-ergic pathway were involved in the vagally induced non-cholinergic, non-adrenergic motor response these substances should mimic the vagally elicited responses. This was also found to be the case since leu- and met ENK i.a. elicited a pyloric contraction and a gastric relaxation. These responses were completely abolished by Nal, an effect which seemed to be selective for ENK since the Ach induced motor responses were unaffected by Nal-infusion. Intraarterial infusion of other opiate receptor agonists such as Mo and ENK-amide elicited similar responses as met and leu-ENK in the pylorus, but instead caused a slow increase of the gastric tone These effects could be blocked by equimolar bolus doses of Nal. On a molar basis ENK

was about 20 times more potent than Mo although the Mo-effect was more longlasting. In most other systems the opposite is observed where Mo is found more potent (Snyder & Childers, 1979 Ambinder & Schuster 1979). There is, however evidence for multiple opiate receptors. A certain subclass of receptors, delta receptors, which have a high affinity for ENK and low affinity for opiate alkaloids including the opiate antagonist Nal (Lord et al., 1977 Terenius 1977 Kosterlitz & Leslie 1978) has been suggested. Since ENKs are very unstable in plasma, it has sofar not been possible to establish if ENK is released to the venous blood from the pylorus on efferent vagal nerve stimulation (Dupont 1977 Sullivan et al 1978)

The rapid enzymatic degradation of ENKs in plasma suggests that the humoral transport of ENK to a distant site of action is less likely (Teschemacher et al., 1978) However the results of the present study indicate that ENK-ergic nerve fibres are involved in the control of pyloric contractility Although several of the present findings strongly indicate that ENK is a neurotransmittor in the gastrointestinal tract more experimental work has to be done before this can be fully established.

Besides the rich ENK-ergic innervation of the pyloric region the immunohistochemical studies also demonstrated VIP-containing nerve terminals and VIP-immunoreactive nerve cell bodies in the pyloric region as well as VIP-like immunoreactivity within vagal axons Therefore it was also of interest to study the effect of VIP on the pyloric and gastric motor function (IV)

Close i.a. injection of VIP to the pylorus and stomach caused a prompt increase of the transpyloric flow and a relaxation of the stomach. These effects could not be blocked by neither Atr nor Hex. The VIP-induced gastric relaxation which was similar to the receptive relaxation was of a rather long duration (10 – 15 min) whereas the pyloric relaxation was much shorter in duration (4 – 5 min) These studies confirm that VIP seems to have a general relaxatory effect on the gastrointestinal tract (Morgan et al., 1978) but that the duration of the response may vary between different parts of the gastrointestinal tract A similar prolonged inhibitory response of the stomach to VIP injection has previously been shown by Eklund et al (1979).

VIP has previously been shown to relax the cardia sphincter of the opossum (Rattan, 1977) and vagal nerve stimulation has also been shown to significantly increase the venous plasma VIP concentration (Fahrenkrug et al., 1978 b) These authors have therefore suggested that VIP is a neurotransmitter in the gastrointestinal tract (Fahrenkrug, 1979) probably mediating the non-cholinergic non-adrenergic receptive relaxation It is also possible that the vagally induced pyloric relaxation is mediated via a similar mechanism

In conclusion It was found that 1) the i.a. injection of met and leu ENK caused a pyloric contraction and a gastric relaxation. The effects were possible to block or reduce with Nal in a dose dependent manner 2) ENK-amide on the other hand caused a contraction of both the stomach and the pylorus, and these effects could also be antagonized by Nal. 3) The pyloric contraction elicited on *efferent* cervical vagal stimulation was inhibited by Nal in a dose dependent manner 4) The second peptide VIP with a similar morphological distribution as ENK, caused a pyloric and gastric relaxation at i.a. injec

tion (Fig. 5) The results of the immunofluorescence and physiological studies indicate that ENK and VIP may serve as neurotransmitters in the pylorus and stomach. (Fig. 6)

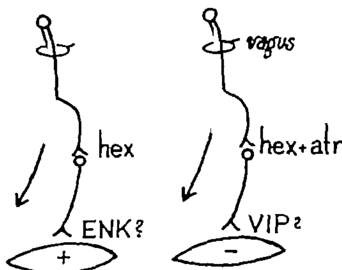


Fig. 6 Hypothetical interpretation of the present immunohistochemical studies of the pylorus in combination with the findings in experiments on the transmission mechanism of the vagal control of the feline pylorus.

Immunohistochemistry of human vagal nerve (V)

Immunohistochemical studies were performed on human vagal nerves (V) in order to investigate whether peptidergic neurotransmission is involved in the motor control of the pylorus and stomach in *man*. A high number of SP fibres, medium numbers of ENK fibres, and a few VIP fibres were found in these vagal nerves examined, that is the thoracic nerve trunk, anterior subdiafragmatic vagal nerve and the nerve of Latarjet (V). Thus, the vagal nerve, both in man and in cat was demonstrated to be a heterogeneous nerve containing several different peptide systems in addition to cholinergic and adrenergic systems. (Muryobayashi 1968, Burnstock 1972, Lundberg et al. 1976 and V). The present study indicates the presence of three peptides, SP, VIP and ENK, in the axons of the human vagus as revealed by immunohistochemical studies of nerves ligated *in vivo* and later extirpated during surgery. Since the peptides accumulate centrally to the ligation a peripherally directed axonal transport seems probable.

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seems suitable for such an action. The physiological stimuli for this reflex are not established but an increased discharge of vagal afferent fibres has been demonstrated on stimulation of either chemo- or mechano-receptors of the intestine stomach and oesophagus (Andrew 1957 Iggo, 1957 Harper et al., 1959 Paintal, 1954)

The integrated gastric response to vagal stimulation seems meaningful for the digestion of food It induces a receptive relaxation and a concomitant closure of the pylorus. The low threshold vagal fibres facilitate movements of the stomach, which are necessary to mix the food with acid and pepsinogen, also under vagal influence (Martinson, 1965 b). All these activities require an increased blood supply regulated by vagal nerves. The non-cholinergic non-adrenergic transmission involved in some of these mechanisms is still unclear but it is interesting to note that an infusion of ENK into a gastric artery has been shown to increase acid and pepsinogen secretion and the mucosal blood flow (Konturek et al., 1978) and to further cause a pyloric contraction and gastric relaxation, as shown in the present series of experiments.

GENERAL CONCLUSIONS

The following conclusions were drawn on the basis of the observations made

1 The vagal nerves in cat contain both excitatory and inhibitory fibres to the pylorus. Both effects seem to be mediated via non-cholinergic, non-adrenergic mechanisms in contrast to the cholinergic gastric contraction. A vago-vagal excitatory reflex to the pylorus was elicited by afferent vagal nerve stimulation together with a vago-vagal gastric relaxation.

2. Within the feline pylorus numerous nerve fibres with ENK-like immunoreactivity both in the circular muscle layer and the myenteric plexus were demonstrated Many cell-bodies with ENK-like material were demonstrated in the myenteric plexus. The pyloric region was far more densely innervated with ENK-positive nerve fibres than the other parts of the stomach. Within the circular muscle layer VIP-immunoreactive fibres had a distribution very similar to the ENK-positive nerves.

3 Local intraarterial infusion of the opiate antagonist naloxone blocked the pyloric contraction obtained on efferent vagal nerve stimulation in a dose dependent manner VIP or ENK injected i.a. resulted in a gastric relaxation while the two peptides had antagonistic effects on the pyloric motility VIP inducing relaxation and ENK contraction These results suggest a vagal control of the feline pylorus and stomach which is mediated by these peptides

4 The presence of axons in the human vagus with SP ENK and VIP-like immunoreactivity was demonstrated

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SUPPLEMENTUM 487

Diet and Muscle Metabolism in Man

with reference to fat and carbohydrate utilization and its regulation

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CONTENTS

The present thesis is based on the following papers which will be referred to by their Roman numerals

I. JANSSON E. Acid soluble and insoluble glycogen in human skeletal muscle Acta Physiol Scand. Accepted for publication

II JANSSON E On the significance of the respiratory exchange ratio after different diets during exercise in man. Acta Physiol Scand. Accepted for publication

III JANSSON E. & KAIJSER, L. Effect of diet on the utilization of blood-borne and intramuscular substrates during exercise in man. Acta Physiol Scand. Accepted for publication

IV JANSSON E. & KAIJSER, L. Effect of diet on muscle glycogen and blood glucose utilization during a short term exercise in man

V JANSSON E. & KAIJSER, L. Leg citrate metabolism at rest and during exercise in relation to diet and substrate utilization in man

In addition, some unpublished results are included.

INTRODUCTION

Already in 1939 studies by Christensen and Hansen suggested that a change in the proportions of fat and carbohydrates in the diet would influence the relative oxidation of these two types of substrates in the working muscles as judged from the respiratory exchange ratio (R) By using the percutaneous muscle biopsy technique Bergström et al (1967) and Gollnick et al (1972) demonstrated a greater intramuscular glycogen content and a greater decrease in glycogen during exercise after a carbohydrate rich than after a fat rich diet. The present series of studies was undertaken in order to further investigate the relationship between the composition of the pre exercise diet and the substrate utilization during exercise thus suggested.

In the methods conventionally used for the analysis of intramuscular glycogen, perchloric acid extraction of the muscle tissue is a critical step. Thus variations in acid solubility of the glycogen present in the muscle is a potential source of error in these analyses To evaluate the magnitude of this analytical problem a methodological study was performed (I)

R is a basic parameter used for evaluating a possible effect of the diet on the muscle substrate utilization during work in the present as well as in previous studies However R gives only an indirect estimation of muscle substrate metabolism, since the gas exchange over the lungs represents a mean for the whole body Furthermore extreme diets may cause general alterations of the acid base balance which would influence the release of CO_2 from the bicarbonate pool and thereby change the R. Thus in a second study acid base parameters and the relationship between R and the respiratory exchange ratio as measured directly over the leg (RQ_L) were studied during a submaximal exercise after two diets differing with respect to the relative proportions of fat and carbohydrates This was done to evaluate if R gives a relevant estimation of the proportions of fat and carbohydrate oxidation in working skeletal muscle even after extreme diets

To obtain a more detailed information on the contribution of blood-borne and intramuscular substrates during exercise after a fat and after a carbohydrate rich diet healthy subjects were studied during a long (III) and a short (IV) term submaximal exercise after the same types of diet as in the study just described. Thus in addition to intramuscular glycogen intramuscular triglycerides and blood-borne substrates such as free fatty acids (FFA) and glucose might also be influenced by the diet. An increased plasma concentration of FFA is probably at hand after a fat rich and carbohydrate poor diet and the utilization of free fatty acids (FFA) has been found to be related mainly to the plasma concentration of FFA (see e g Hagenfeldt & Wahren 1971) On the other hand a corresponding relationship between utilization and concentration has not been shown or thought to be at hand for glucose or glycogen utilization (cf Randle & Smith 1958 Morgan, Randle & Regen 1959 Newsholme & Crabtree 1979)

Assuming that other factors than the supply of carbohydrates regulate the glycolysis concurrently with a varying availability of FFA the rate of fatty acid oxidation could possibly regulate the glycolysis by the action of metabolic intermediates on enzymatic steps (in the glycolysis) according to a hypothesis presented by Garland Randle & Newsholme (1963) Citrate was thus proposed to be a key-intermediate This was based upon the findings that during an increased supply and oxidation of fatty acids in the perfused rat heart, an inhibition of the phosphofructokinase (PFK) reaction was found synchronously with an increased citrate content and that the PFK reaction in vitro was inhibited by citrate (Garland Randle & Newsholme 1963) Therefore the possibility of a citrate mediated regulation of the glycolysis due to an altered rate of fat oxidation was investigated by studying the leg citrate metabolism and its relation to the concomitant leg substrate utilization during exercise in the two dietary situations (V)

MATERIAL AND METHODS

Subjects In 11 18 mal and 26 femal healthy ubjects participated Mean and SD f r various background variabl are given in Tabl 1 All subjects we inf rmed on the procedur and purpose of the experiment and on po sible isks bef gi ing an o al consent The subject we fre t withdraw their consent to participate t any time The tudy was approved by the Ethical ommittee f the Karolin ka hospital

Experimental procedure (I) Muscl glycogen analyse perf rmed on tissue ubjected to ext action by perchloric acid pri to the analyses wer comp ed to rr sponding analyse on tis ue without such pretreatment 99 biopsies covering wid range f glycogen concent tions were analysed The muscl mat rial was taken from the biopsies f tudi III and IV

Experimental procedure (II V) Each subject was tudied at rest and during rgomet r bicy l exercise t 65% f maximal xygen uptake ($\dot{V}O_2$ max) on two occa ion The $\dot{V}O_2$ max was d t rmined bef rehand with the subj cts on normal di t The exercise time wa 25 min in tudi II III and V and 6 min in tudy IV The fi st expe iment was p eceded by 5 day on fat rich and carbohydrat poo di t (~70% f t 5% p t in and 25% arbohydr te ; 8 MJ/day) Thi was f llowed immediately by 5 day on a carbohyd t ich and f t poo di t (~70% carbohyd at 10% f t and 20% protein; 8 MJ/day) and an identi l experimental procedure Detail on the di t have been given in study II On the days f the experiment the subjects came to the labor tory aft light morning meal (~800 kJ) with the same composition as the preceding diet

Tabl 1 Age heigh weight maximal oxygen uptake ($\dot{V}O_2$ max) and maximal heart rate (max HR) for the subjects in study II III and IV[1] (n number of subjects)

	Age yrs	SD	Height cm	SD	Weight kg	SD	$\dot{V}O_2$ max l min	SD	Max HR beats min[-1]	SD	( )
Study II	22	2	79	11	67	14	2 88	0 62	188	9	7 (2 5)
Study III	25	3	1 2	8	66	7	3 00	0 65	190	6	20 (9 11)
Study IV	24	3	1	8	67	8	3 0	0 53	190	9	(4 3)

[1] The sampling for studies I and was performed imultaneously on the subjects in studie III and IV
In tudies III and V in addi ion to the presnt experimental procedure two groups of subjects were studied.
I study III Hb analyse were performed in subjects fter the f and the carbohydrate diet and in study oxygen uptake and were analysed in 4 subjects after the two diets These subjects (mal and females) were of same age he ght we gh and physical fitness as the other subject

Table 2. Analyses performed in the present series of experiments

Analyse	Technique	Reference	CV[1] at low values of variable	CV[1]	CV[1] at high values of variable	Analyses performed in the different studies I	II	III	IV	V
Gas analyses										
oxygen	volumetric	Scholander 1947	0.6		0.5					
carbon dioxide		—	0.8		0.8					
Blood analyses										
FFA (plasma)	radiochemical (^{63}Ni)	Ho 1970	2.7		2.0					
glucose	spectrophotometric, glucose-oxidase	Huggett & Nixon 1957		1.1						
β-hydroxybutyrate	fluorometric enzymatic	Hoo se & Nevel 1970	4.3		1.1					
acetoacetate	—	Lowry & Passonneau 1973		1.2						
pyruvate	—	Bücher et al 1962 (ad modum)	4.6		2.2					
lactate	—	Hohorst 1962 (ad modum)	3.4		0.8					
^{14}C-palmitate	liquid scintillation counting	see Olsson et al 1975 1977		2.4						
oxygen saturation	spectrophotometric; IL 182 CO-Oximeter Inst Lab Inc USA	Drabkin & Smidt 1945	0.5		0.1					
haemoglobin				0.8						
pH	BMS 3 Mk 2 Radiometer Copenhagen; electrodes for pH and PCO_2	Severinghaus & Bradley 1958		0.1						
PCO_2			1.9		1.3					
oxygen concentration	manometric	Peters & van Slyke 1932	3.6		1.5					
carbon dioxide conc.		—	1.1		0.7					
Muscle analyses[2]										
glycogen	acid hydrolysis into glucose residues	Lowry & Passonneau 1973	7.7		12.4					
triglyceride	hydrolysis into glycerol and fatty acids	Chernick 1969 Essén 1978		17.0						
glucose	fluorometric enzymatic	Lowry & Passonneau 1973								
glycerol	—	—								
muscle preparation[3]	neutralized perchloric acid extract	see Essén 1978								
[illegible]	fluorometric enzymatic	Lowry & Passonneau 1973	7.8		7.4					
lactate	—	Karlsson 1971	13.1		14.1					
glucose-6-phosphate	—	—	12.4		6.3					
α-glycerophosphate	—	Lowry & Passonneau 1973	8.3		7.1					

1) Coefficient of variation ($\frac{SD\ diff}{\bar{X}} \cdot \frac{1}{\sqrt{2}} \cdot 100$) was based on duplicates from the same blood and muscle sample. In the case where the analyses were performed on extracts of the blood or muscle samples the duplicates represent two extracts each subjected to double analysis (X) i.e. $X \langle X_1, X_1 \rangle$ $X_2 \langle X_2, X_2 \rangle$. The differences were calculated as $\bar{X}_1 - \bar{X}_2$.

2) All muscle analyses were performed on freeze-dried samples.

3) All muscle analyses of metabolites were performed on perchloric acid extracts whereas both glycogen and triglyceride analyses were performed on separate samples.

Assuming that other factors than the supply of carbohydrates regulate the glycolysis concurrently with a varying availability of FFA the rate of fatty acid oxidation could possibly regulate the glycolysis by the action of metabolic intermediates on enzymatic steps (in the glycolysis) according to a hypothesis presented by Garland Randle & Newsholme (1963) Citrate was thus proposed to be a key-intermediate This was based upon the findings that during an increased supply and oxidation of fatty acids in the perfused rat heart, an inhibition of the phosphofructokinase (PFK) reaction was found synchronously with an increased citrate content and that the PFK reaction in vitro was inhibited by citrate (Garland Randle & Newsholme 1963) Therefore the possibility of a citrate mediated regulation of the glycolysis due to an altered rate of fat oxidation was investigated by studying the leg citrate metabolism and its relation to the concomitant leg substrate utilization during exercise in the two dietary situations (V)

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Table 1 Age heigh we ght, maximal oxygen uptake ($\dot{V}O_2$ max) and maximal heart te (max HR) for the subjects in tudy II III and IV[1] (n number of subjects)

	Age yrs		Heigh cm		Weight kg		$\dot{V}O_2$ max l min^{-1}		Max HR beats min^{-1}		n (♀)
		SD	M	SD	M	SD		SD		SD	
Study II	22	2	78	11	67	14	2 88	0 62	188	9	7 (2 5)
Study III	25	3	172	8	66	7	3 00	0 65	190	10	20 (9 11)
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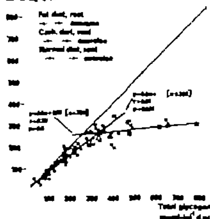


Fig 1 Relationship between glycogen concentration in PCA pretreated muscl samples (PCA insolubl glycogen) and glycogen in ampl without acid pretreatment (total glycogen) The larger symbol represent samples where PCA insolubl and t tal glyc gen were determined imultaneously

Intramuscular substrates and metabolites (III IV V; Fig 2) The muscle glycogen concentration was lower both at rest and during exercise after the fat than after the carbohydrate diet and the decrease in glycogen concentration during exercise tended to be lower after the fat diet. The interindividual difference in glycogen decrease was larger after the carbohydrate than after the fat diet. The intraindividual variation in glycogen concentration before and after exercise was larger after the carbohydrate diet (III) However it could not be concluded whether there really was a large variation between subjects or legs in decrease in the whole muscle or if the differences were caused e g by variations in glycogen between different parts of the muscle. The large variation could anyway have contributed to the lack of a significant difference between the diets in glycogen decrease Support for the idea that the decrease was actually smaller after the fat than after the carbohydrate diet was found in the fact that the muscle lactate accumulation as well as the lactate release over the legs were smaller both in the early phase and at the end of exercise

There was no significant decrease in intramuscular triglyceride concentration during exercise after any of the diets. This may be due to the large variations in fat distribution in the muscle tissue (Carlsson, Ekelund & Fröberg 1971 Essén 1978) Other studies have shown a breakdown of muscle triglycerides during exercise of both short and long duration (Essén 1977) and numerically the difference between triglyceride concentrations at the end and at the start of exercise was negative after the fat and positive after the carbohydrate diet. This could be expected if the utilization of intramuscular triglycerides is greater after a fat than a carbohydrate diet.

The muscle citrate concentration was higher after the fat than after the carbohydrate diet at rest and 6 min of exercise whereas no difference was found at the end of exercise The glucose-6-phosphate (G-6-P) concentration was higher at rest, tended to be higher at 6 min of exercise and was lower at the end of exercise after the fat than after the carbohydrate diet.

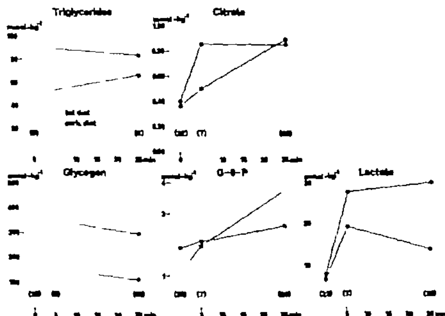


Fig 2 Muscl concent tions f triglyc rid glyc gen itrat glucose-6-phosphate (G-6-P) and lact t t rest and ft r 6 and 25 min f exer ise ft r the f t (filled i les) and after the carbohydrat (pen circles) di t The number f ubject for each point f time and analysis given within parenthesi
1)The glycogen v lues t 6 and 25 min we calculated f llows mean pre ex is value (23) ubtract d by median decrease over the 6 min wo k pe iod (6) and mean p xercis value (23) subt acted by median decreas ove he 25 min work period (16) respectively

Blood-borne substrates and metabolites (III IV) During exercise the estimated leg blood flow was of similar magnitude in both dietary situations and consequently the r between diets in uptake or release was mainly the same as estimated from the a-fv differences alone However at rest due to the higher estimated leg blood flow after the fat diet, the uptake/release was not directly reflected by the a-fv differences (see V)

The plasma FFA concentration as well as the FFA uptake were higher after the fat than after the carbohydrate diet both at rest (unpublished) and during exercise (Fig.3)

It has repeatedly been demonstrated that the uptake of FFA from blood to muscle is correlated with the arterial FFA concentration and that the fractional extraction is unaltered or slightly decreased when the FFA concentration increases (e g Hagenfeldt & Wahren 1971) In contrast to this in the present study the fractional extraction during exercise tended to be higher at an increased FFA concentration. Thus the gross extraction of FFA was on the average 82% greater after the fat than after the carbohydrate diet whereas the arterial FFA concentration was only 66% higher The present finding could possibly indicate a stimulation of intracellular fat utilization in exercising muscle after a fat rich diet in excess of the effect of an increased availability

The arterial glucose concentration was lower after the fat than after the carbohydrate diet both at rest and during exercise (Fig 3) The uptake of glucose at rest was also lower but during exercise no significant diet related difference was found. In fact there was a tendency to greater uptake after the fat diet (Fig 3)

The glucose extraction during exercise was not lowered after the fat diet in spite of an increased FFA extraction and presumably also an increased FFA oxidation. In contrast it should be noted that an inverse correlation between FFA and glucose extraction has been observed for the myocardium (Lassers et al 1971) However the present finding is in agreement with those of previous studies on the effect on the skeletal muscle metabolism of FFA-lowering by nicotinic acid which did not alter the glucose extraction by the working forearm (e g Kaijser et al 1978) On the other hand

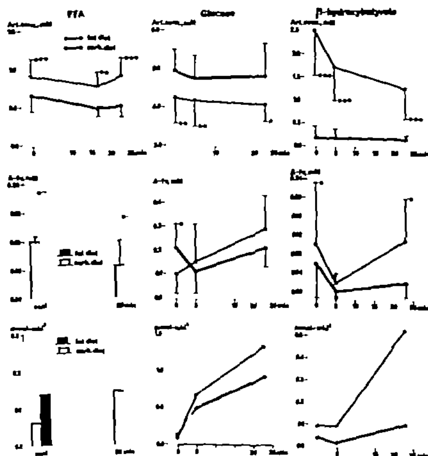


Fig 3 Art ial oncentra ions art rial femor l venous (a-f) diff renc (gros ext action for FFA) and uptake of plasma free f tty acids (FFA) blood glucos and β-hydroxybutyr t t rest and during exercise f the f t (filled line / ircles/bar) and aft the bohyd at (open line / ircl /bars) di t

there was a tendency towards an inverse relationship between the glucose extraction and the glycogen concentration in the muscle after the fat diet. This is in agreement with the observation of a higher glucose extraction over one leg with low muscle glycogen than over one with high muscle glycogen content (Gollnick et al 1980)

The arterial β-hydroxybutyrate concentration was higher after the fat diet both at rest and during exercise (Fig 3) At rest there was a significant uptake only after the carbohydrate diet and at the end of the exercise only after the fat diet

β-hydroxybutyrate may be utilized in the oxidative metabolism. However the exchange of acetoacetate over the exercising leg was not measured. Consequently it is not known whether or not the β-hydroxybutyrate was fully oxidized However if so the contribution to the total oxidative metabolism could have been about 8% after the fat diet as compared to 2% after the carbohydrate diet and could thus have contributed only moderately to the difference between the diets in substrate utilization.

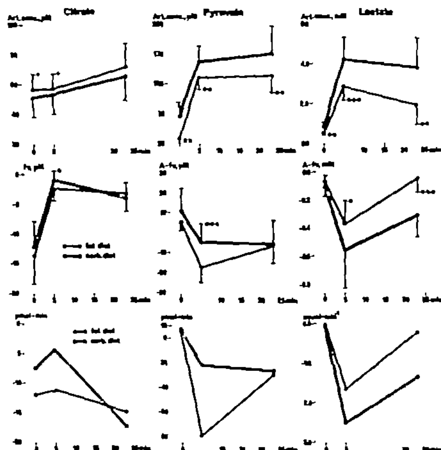


Fig 4 Arterial oncentr tions art ial femo al venous (a-fv) diff rences and uptake f blood it t pyruv te and lactate afte the f t (filled line and ircles) and after the carbohydr te (open line and ircl) di t

The arterial lactate and pyruvate concentrations were lower after the fat than after the carbohydrate diet both at rest and during exercise (Fig 4) At rest the lactate release did not differ between the diets but during exercise it was smaller after the fat diet (Fig 4) Pyruvate was taken up at rest whereas after 5 min of exercise there was a release after both diets but the release was larger after the fat diet in contrast to the situation for the lactate release which was smaller after the fat diet At the end of exercise no significant difference between the diets in pyruvate release was found.

The arterial citrate concentration was higher after the fat than after the carbohydrate diet both at rest and during exercise There was a net release of citrate after both diets both at rest and during exercise At rest and 5 min of exercise the citrate release was greater after the fat diet whereas at end of exercise no diet related differences were found

Notably the greater release of pyruvate and citrate found after the fat than after the carbohydrate diet, occurred together with a lower lactate release This indicates an inhibition of the pyruvate dehydrogenase (PDH) reaction, directly or indirectly coupled to the increased citrate formation.

GENERAL DISCUSSION

Total leg metabolism. The simultaneously measured extractions of oxygen and blood-borne substrates makes it possible to estimate the relative net contribution of these substrates to the total oxidative metabolism and if R is taken into account, also of the fat and carbohydrate oxidation, respectively (Fig 5)

After 25 min of exercise FFA contribution to the total oxidative metabolism was 24% after the fat diet and 14% after the carbohydrate diet. The remaining fat oxidation, 39% of the total after the fat and 13% after the carbohydrate diet must consequently have been covered by plasma and intramuscular triglycerides Assuming an active muscle mass in the legs of 6 kg (cf Essén, Hagenfeldt & Kaijser 1977) the pre exercise concentrations after both diets suggested a 10-20 times greater supply of intramuscular triglycerides (90 7 mmol x kg^{-1} after the fat diet and 50 4 mmol x kg^{-1} after the carbohydrate diet) than that required to cover all the remaining fat oxidation but in no dietary situation was a significant decrease noted The tendency towards a decrease in concentration after the fat but not after the carbohydrate diet may suggest,however that more intramuscular triglycerides are utilized after the fat diet. Nothing is to our knowledge known about the plasma triglyceride extraction during exercise after different types of diet, although after a normal mixed diet it could cover at the most 25% and probably far less of the oxidative metabolism (Olsson et al 1975)

The glucose extraction at 23 min of exercise was equivalent to 27 and 21% of the oxidative metabolism after the fat and the carbohydrate diet respectively but when subtracting the lactate release the glucose contribution would change to 25% after

there was a tendency towards an inverse relationship between the glucose extraction and the glycogen concentration in the muscle after the fat diet. This is in agreement with the observation of a higher glucose extraction over one leg with low muscle glycogen than over one with high muscle glycogen content (Gollnick et al 1980)

The arterial β-hydroxybutyrate concentration was higher after the fat diet both at rest and during exercise (Fig 3) At rest there was a significant uptake only after the carbohydrate diet and at the end of the exercise only after the fat diet.

β-hydroxybutyrate may be utilized in the oxidative metabolism. However the exchange of acetoacetate over the exercising leg was not measured Consequently it is not known whether or not the β-hydroxybutyrate was fully oxidized. However if so the contribution to the total oxidative metabolism could have been about 6% after the fat diet as compared to 2% after the carbohydrate diet and could thus have contributed only moderately to the difference between the diets in substrate utilization.

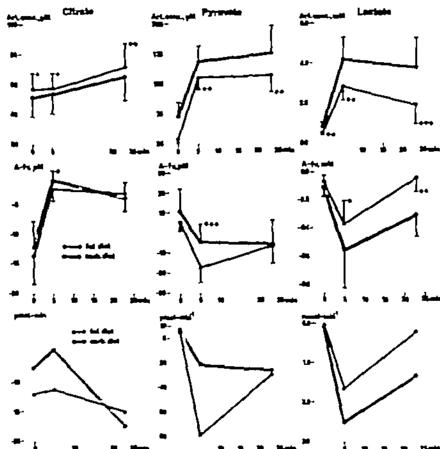


Fig 4 Art rial on ent tions art ial-femoral venous (a-fv) diffe ence and uptake f blood itrat pyruv t and lactat ft the fat (filled line and ircl) and after the carbohydrat (open lines and rcles) di t

Thus the general picture suggests that (1) a fat rich diet increases the relative contribution of fat to the oxidative metabolism during exercise as compared to a carbohydrate rich diet, (2) the increased fat utilization is to a great extent covered by plasma FFA and (3) the simultaneous decrease in carbohydrate utilization concerns muscle glycogen rather than glucose

At rest the situation seemed quite different as compared to that during exercise There was an uptake of substrates in excess of the oxidative utilization and this indicated a net increase of the stores of especially fat and to some extent carbohydrates

Aspects on citrate measurements A number of technical problems are associated with the estimation of muscle citrate concentrations and some of them will be discussed below Firstly a rapid increase of muscle citrate occur at cessation of work (unpublished) Most biopsies were taken and frozen within 10 after the last muscle contraction. The citrate increase in the muscle over this period of time is difficult to estimate. However an increase of about 30% from the 10th to the 20th s after a submaximal exercise with the subjects on a normal diet has been observed (unpublished)

Secondly human skeletal muscle is composed of a mixture of type I (red) and type II (white) muscle fibres in a ratio of about 1:1 but a relatively large variation may occur within an individual and within a particular muscle This together with the fact that the citrate content is approximately twice as high in the slow twitch fibres as in the fast twitch fibres in human muscle both at rest and immediately after exercise contributes to the methodological error of citrate estimations (cf Essén 1978)

Thirdly animal studies have shown an accumulation of citrate in red but not in white skeletal muscle with an increased rate of fat oxidation during exercise (Rennie Winder & Holloszy 1976) Thus to evaluate in greater detail the role of citrate in the regulation of muscle substrate utilization analyses should be carried out on individual red and white muscle fibres However this was not done in the present study

Finally the critical concentration of citrate for the regulation of glycolysis is the concentration in the sarcoplasm and not the total muscle citrate Excess citrate produced in the mitochondria will be transported to the sarcoplasm and from there to the extracellular space The release of citrate from the muscle may therefore better than the total muscle concentration reflect the sarcoplasmic citrate.

The present study demonstrated a net release of citrate from the leg to the blood both at rest and during exercise after both diets The turnover of citrate in relation to its concentration in blood and muscle was high which allows rapid changes in concentrations which in turn is a prerequisite for citrate s regulatory importance A good correspondence between the estimated citrate release and the muscle citrate concentration was found after both diets as well as diet related differences. Thus in spite of the great techniqual difficulties described above in estimating the actual sarcoplasmic citrate concentration the found differences between the diets support citrate being of regulatory importance for fat and carbohydrate metabolism.

Metabolic regulation in relation to diet Background. (Fig 6) According to recent theories (Newsholme & Crabtree 1979) glycolysis in skeletal muscle may be divided into two separate pathways (1) glycolysis-from-glycogen and (2) glycolysis-from-glucose, on the basis of different flux generating steps (i e the reaction where the flux through the pathway is initiated) The capability of the muscle to control these two flux generating reactions might differ Thus phosphorylase (PL) catalyses the flux generating step for the flux from muscle glycogen (the pathway substrate) The PFK reaction has a potential to regulate the activity of PL via the G-6-P concentration. However only PLb and not PLa is thought to be under the control of PFK (Morgan & Parmeggiani 1964) and PFK may therefore only have a partial capacity to regulate the flux generating reaction. The substrate for the second pathway glycolysis-from-glucose is under debate. It has been argued (Newsholme 1977) that the pathway substrate is in fact liver glycogen and if so glucose would be regarded as a metabolic intermediate It has further been considered unlikely that muscle PFK, by varying the G-6-P level which in turn influences the hexokinase (HK) reaction in the muscle could regulate the suggested flux generating reaction in the liver The glucose transport over the muscle cell membrane has instead been proposed to be a reaction that could influence the hepatic phosphorylase via (blood) glucose itself (Newsholme & Crabtree 1979) Thus according to the theories now presented citrate by its influence on the PFK reaction might have a potential to regulate only the rate of glycolysis-from-glycogen and only via PLb inhibition. In an attempt to apply this approach to the findings in the present study the resting 5 min and 25 min situations will be discussed separately

Rest The leg citrate release as well as muscle citrate and G-6-P were found to be higher after the fat than after the carbohydrate diet. An estimation of the quantitative relationship between total rate of glycolysis after the diets as judged from R and exchange of lactate and pyruvate over the legs suggests a lower rate of glycolysis after the fat diet. The measured substrate utilization revealed a lower glucose and

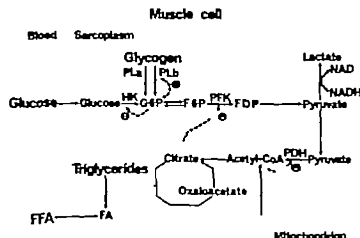


Fig 6 A schematic illust r tion f metabolic pathways involved i the deg ad tion of fat and carbohydr te in the muscl II Cont 1 of the glycolysi and pyruv t oxidation by itrat and ac tyl-CoA.

a higher FFA uptake after the fat than after the carbohydrate diet. This supports the idea of a lower rate of glycolysis as proposed above and consequently it appears possible that the inhibition of glycolysis could be mediated through the increased muscle citrate acting via a PFK inhibition, an increased G-6-P level and an inhibition of PLb.

5 min exercise. The situation at 5 min of exercise showed some features in common with that at rest. Both citrate release over the leg and the muscle citrate concentration were higher after the fat than after the carbohydrate diet and the G-6-P concentration showed the same tendency. The total rate of glycolysis, as judged from R, lactate, pyruvate and alanine release, was lower after the fat diet also during exercise. Support for this was found in the lower ($p < 0.2$) muscle glycogen decrease and lower muscle lactate increase after the fat diet. However, the glucose uptake did not differ between the diets. Accordingly, the increased muscle citrate and G-6-P concentrations are consistent with the theory of a citrate mediated inhibition of glycolysis including a control of the flux generating step through the G-6-P level. The flux of glucose through the glycolysis seemed not to be different in the two dietary situations as could be expected if PFK is not a regulatory enzyme in the glycolysis-from-glucose (cf Newsholme & Crabtree 1979). Thus, it is suggested that the glycolysis is partly under control of the PFK reaction. The observation of a greater release of pyruvate by the leg after the fat than after the carbohydrate diet in spite of a lower lactate release, indicates however, an imbalance between the rate of glycolysis on one hand and the NADH availability and PDH activity on the other. One factor contributing to this suggested imbalance might be an increased rate of glycolysis caused by an increased conversion of PLb into PLa. This view is supported by our earlier finding (Jansson, Bjömdahl & Kaijser 1980) of increased plasma catecholamine levels after a similar fat diet with ensuing β-receptor stimulation and thereby increased formation of cyclic AMP, which might in turn stimulate a conversion of PLb into PLa (Danforth & Helmreich 1964, Sutherland, Oye & Butcher 1965). Support for a difference between the diets in NADH availability was found in the lactate/pyruvate ratio in the blood, which was lower after the fat diet. Furthermore, animal studies have demonstrated a PDH inacti-inactivation after a high rate of fat oxidation (Hennig, Löffler & Wieland 1975).

15 min exercise. At this time diet related differences were found neither for the citrate release nor for the muscle citrate concentration. However, the total rate of glycolysis, estimated as described above, was still lower after the fat diet. The measured substrate utilizations partly support this (higher FFA uptake, tendency to lower decrease in glycogen, lower increase in lactate and a tendency to higher glucose uptake). The G-6-P concentration was found to be lower after the fat diet. Thus, no correspondence was found between muscle citrate accumulation and the rate of glycolysis at the end of exercise. However, after a work period of this duration it cannot be excluded that the lower rate of glycolysis after the fat diet would merely be related to very low glycogen concentrations. Thus, in recent studies on short term intense exercise and lactate accumulation it has been suggested that the glycogen concentration

would influence the rate of glycogenolysis because the PL may not be saturated with its substrate (Klausen & Sjøgaard 1980 I Jacobs and J Karlsson personal communication)

The finding of similar magnitudes of both citrate release and muscle citrate concentration when comparing the dietary situations indicated that the rates of citrate synthesis were the same The latter is in turn dependent on the supply of acetyl-CoA and oxaloacetate The similar citrate magnitude might thus be explained as follows:

The amount of acetyl-CoA, as derived from fatty acids was probably larger after the fat than after the carbohydrate diet. However the amount of acetyl-CoA as derived from carbohydrates was possibly markedly reduced after the fat diet because of the very low levels of glycogen that were at hand. The supply of oxaloacetate might as well have been reduced Due to this it is possible that neither the total supply of acetyl-CoA nor the rate of citrate synthesis differed between the diets or that a possibly reduced supply of oxaloacetate was a limiting factor for the citrate synthesis after the fat diet.

Female versus male subjects (Fig 7 Table 3) The group of subjects was not a real random sample which actually is a prerequisite for making a relevant comparison between the sexes. However in spite of this a rough comparison was made Thus among the background variables height, weight, VO_2 max and the relative proportions of different muscle fibre types in m. quadriceps femoris (unpublished) differed significantly between the sexes There was a higher proportion of type I fibres in the quadriceps muscle of the females whereas in earlier studies on sex differences in relative fibre proportions a lower percentage of type I fibres in females (Komi & Karlsson 1979) or no differences at all (Hedberg & Jansson 1976) have been observed. The general picture given by certain metabolic variables (see Fig 6) indicated a tendency towards a relatively higher fat oxidation in the female than in the male subjects as indicated e g by the tendencies towards lower R and higher oxygen uptake in relation to VO_2 max. Also a possible sign of higher activities in the

Table 3 Age, height, weight, maximal oxygen uptake (VO_2 max) and maximal heart rate (max HR) for the 5 male and female subjects and the relative proportions of the different muscle fibre types in % of the males and in % of the female subjects

Variable	Males	SD	Females	SD	Males/females ratio	Significance of the male-female difference
Age, yr	24	3	2[illegible]	3	[illegible]	
Height, cm	[illegible]		65	7	1 [illegible]	0 001
Weight, kg	73	6	6		1	0 [illegible]
VO_2 max, l/min	3 44		2	39	32	[illegible]
Max HR, beats/min	18		9		0 [illegible]	
Type I fibres[1]		6	56		65	05
Type IIa fibres,	35		34	6 4	39	01
Type IIB fibres				5 5	[illegible]	
Type IIC fibres		9	2 3			n.

[1] the subgroup of subjects on which fibre types were available the sex differences regarding the background variables were of the same magnitude as in the whole sample

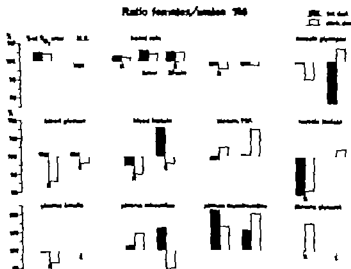


Fig 7 The atio femal /male subject x 100 f the oxygen uptak ($\dot{V}O_2$) and mechanical efficiency (M.E) during exercise heart rate respir tory exchange tio (R) plasma FFA, blood glucos blood lactat muscl glycogen muscl lactate plasma ins lin plasma adrenaline and plasma noradrenaline at est and during exercise afte the f t (filled bars) and afte the rbo- hydrat (open bars) diet The calculations f the diff ent ratios wer based on mean v lues for all subjects on which the diff rent analyse were perf rmed
1)The in lin adrenaline noradrenaline and glycerol values wer taken f om Jansson Bjemdahl & Kaijse (1980) and measurements we e perf rmed on 2 mal and 5 femal subjects only

sympathetic nervous system was found in the tendencies towards higher level of plasma catecholamines higher heart rate and lower plasma insulin concentration in the female subjects. These findings seem reasonable if considering the fact that the females have a relatively larger amount of body fat than the males and that the catecholamines and insulin are both important hormones in the regulation of lipolysis (for ref see Newsholme & Start 1973) To our knowledge only a few studies have been performed with the aim of comparing the sexes with respect to differences in the relative oxidation of fat and carbohydrates One study comparing well trained female and male runners gave no support for a sex related difference in fat metabolism (Costill et al 1979) Alternatively the tendencies towards a relatively higher fat metabolism in the females might exclusively be related to the fact that the females showed a higher percentage of type I fibres which have higher capacity to oxidize fat than type II fibres (e g Essén et al 1975)

CONCLUSIONS

1 Muscle glycogen consists of fractions with different acid solubility The more acid soluble fractions constitute a greater proportion of total glycogen at high than at low concentrations This is a source of error when glycogen analyses are performed on perchloric acid extracted tissue

2. The respiratory exchange ratio (R) as measured at the of a 25 min submaximal exercise does very likely estimate the proportions of fat and carbohydrate oxidation in skeletal muscle both after a fat rich and after a carbohydrate rich diet.

3 A fat rich diet increases the relative contribution of fat to the oxidative metabolism during submaximal exercise This increase is, to a great extent covered by fatty acids from plasma The concomitant decrease in carbohydrate utilization concerns muscle glycogen rather than glucose

4 The inhibition of glycolysis seen at rest and during the early phase of submaximal exercise after a fat diet, might be due to increased intramuscular citrate acting on the phosphofructokinase reaction In addition the pyruvate dehydrogenase reaction is possibly inhibited during the fat diet conditions The inhibition of glycolysis, seen at the end of exercise after the fat diet, does not seem to be citrate mediated.

Index f abbreviations

FFA	f ee f tty acid	PDH	pyruvat dehydrogenase
FA	f tty acids	PFK	pho phofructokinase
F-6-P	fructose-6-phosphat	PL	pho phorylase
F-D-P	fructose-1 6-diphosphate	PLa	phosphoryl se
G-6-P	glucose-6-phosphat	PLb	phosphorylase b
HK	hexokinase	R	pirat ry exchange r tio over the lungs
NAD	nicotinamide adenine dinucleotide (oxidi ed f rm)	RQ_L	pi tory exchange rati over the l g as me sured in the blood
NADH	nicotinamid adenine dinucleotide (reduced f rm)		

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FROM THE DEPARTMENT OF ANIMAL PHYSIOLOGY
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ABSTRACT

RIDDERSTRÅLE Y Intracellular localization of carbonic anhydrase in some vertebrate nephrons

The Han son histochemic l method fo carboni anhydrase has been modified by embedding of the tissue in a re in bef r a ctioning *and incubation* While retaining the now generally *accepted validi-ty* and spe ificity of the original method it has several advantages It is simple and rapid and appears to giv unusually sharp localization of the stain The section thickness may be varied to *suit light micr scopy* and *electron mi roscopy* with *resol tion down* to 25 A. The method has b en disc ssed specially with regard to fal e localization.

The intracellular distribution of carbonic anhydras in the kidneys of frogs at nd birds has been examined

As a basis for th histochemi l investigation an ultrastructur l analysis f th avian n phron ha been perf rmed using mainly young swifts Thin tubules are found exclusively in medullary nephrons and th ir cells are of an unusual structure combining high cell thi kness with trong devel pment of the ps acellular rout The avian initial collecting tubule ntains a high proportion of dark c lls containing mainly baso-lateral carbonic anhyd ase The distribution and morphology of dark cell in vertebrate nephrons has been surveyed These cells ar al o compared with oxyntic cells and it i sugge ted that the c lls are r sponsible f r the terminal acidification of urine taking pl ce in th kidney

A simplified terminology fo the vert b ate nephron is suggested

Key-words: c rboni anhydrase nephron histochemistry lectron microscopy acidification

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Distribution of carbonic anhydrase in the frog nephron 1974
Gudmar Lönn rholm & Yvonne Ridd rstråle Acta physiol scand
90 764-778

Intracellular locali ation of carbonic anhydrase in the frog
nephron 1976 Yvonne Ridderstråle Acta physiol scand 98
465-469

Intracellular di tribution of carbonic anhydrase in the rat
kidney 1980 Gudmar Lönnerholm & Yvonne Ridderstråle
Kidney Int 17:2 In press

Ultrastructure nd localiz tion of carboni anhydrase in the
avian n phron 1980 Yvonne Ridderstråle Swedish J gric Res
In p es

The thesis contains a discus ion of the histochemi al methods
 sed (with some new experimental data) a short survey of the
histochemical results and note of the terminology of the
nephron

INTRODUCTION

Following up an idea by Kurata (1953) Häusler (1958) dev loped the first useful histochemical method for carbonic anhydra His method involved th precipitation f cobalt compound at sites alkalized by th enzymati dehydration of carbonic acid in s ctions floated on th surface f th incubation fluid Mainly by adding phosphat to the incubation medium Hansson (1967) improved th method consid rably and was abl to show that the staining could be inhibited by concentrations f ac tazolamide which wer so low that only the specific inhibitory ffect f the drug could be responsible f r the effect He also showed that the staining by his histochemical method agreed well with biochemical data and presented a series of pictures of impressiv beauty howing the carbonic anhydr distribution in everal organs (Hansson 1968)

Various objections to Häusler s and Hans on s methods hav been raised by Fand t al (1959) and Chorg (1973) but specially by Muther (1972 1977) Th critiqu has been r butted by Rosen t al (1972) Lönn holm (1974 1980) and Maren (1980) The result f this discussion ppea s to be that Hansson s method (prop rly used) ha emerged as a valid histochemical method with a high degree of specificity This i the basi and starting point of the f llowing discussion f some variations of the m thod and possible limitations of thes

THE METHODS

Th sev al v ri tions of the method will be dis us ed mainly on the basi of the expe ienc gained by Lönnerholm & Ridd strâle (1974 1980) and Riddorst Ål (1976 1980) and th discu ion will be concentrated on the techniques used i order to adapt the method to lectron mi roscopy

These methods differ mainly with regard to the stage at which the incubation of the sections is performed In the original Hansson method as used f r electron microscopy by Yokota (1969) Cassidy & Lightfoot (1974) Rosen (1974) and Lönnerholm & Ridderstrål (1974) and with Häusl r s method by H psu et al (1966) and Cross (1970) the incubation is performed with freeze-cut sections of glut raldehyde fixed material After washing and treatment with ammonium sulphide the secti ns are embedded in a suitable resin for electron mi roscopy and ultrathin secti ns are cut At some stage counterstaining with osmium lead or uranyl may be used

The staining takes plac in a multicompartment system divided by lipid membranes more or less denatured and disrupted by fixati n fr ezing and sectioning Thes membranes influenc the diffusion not only f the components of the incubation fluid but also of the primary and final reaction products The factors influencing the distribution of the ensuing precipitate are thus very diffi ult to penetrat This is a drawback howeve which th method ha in ommon with most histochemical methods (Pearse 1968) More important are certain p actical consid rations which have been discu sed by Lönnerholm & Ridd rstråle (1974) On of the diffi lties with thi method is that in rder to g t a reasonably coh rent pictu the ultrathin se tions are cut parallel to the surface f the original thick section The precipitate is thickest at the upper surface and thins out suc es ively downwards and di appear compl tely a few microns from the surface The line dividing the st ined f om the unstained part of the section is f irly st aight but the wavy Th distribution of the precipitat and its density in th thin ections are of course distinctly influenced by this circumstanc (Lönnerholm & Ridderstråle 1974 Fig 4) Th interpretation of the picture is thus to a e tain degree subjective I ddition the staining of

for instance mitochondri was rather erratic In a few cases the interior of the mit chondria was all stained except the memb an s in other cases th interior was compl tely unstained

Some of the difficulties encountered appeared pos ible t overcom with suitable minor modifi tions Th erratic appearance of the p ecipitate on the oth r hand we consid red mo e se iou and prompted effo ts aimed t the development of a method where the incubation takes pl e aft r embedding and sectioning for lect on mi oscopy

The obvious difficulty with this approach i that the enzyme may b compl t ly ina tivated by the embedding procedur or that the components of the incubation olution may b excluded from the sites of enzyme ctivity

After extensive t ials it was found that the re in JB-4 (Sorvall and Polyscienc) appeared itabl This is esin produced by polymerization of a mixtur of glycol m thacryl te and polyethylene glycol (Ruddell 1967 b)

The se of thi resin made possibl the dev lopment f a method for light mi roscopy which was convenient and did not equir any peci l appar tus *It resulted in excellent pre rv tion of* cell morphology and gav very harp and highly reproducibl localizati n of the tain Thin sections (down to 0 5 µm) res lted in harp pi ture and resolution to the limit of light mi ros opy

Though the resin i primarily intended fo light microscopy suitable precautions make it possible to cut sections thin eno gh for electron micro copy (Ridd rst Al 1976 1980)

When thi method is used the lipid membran s are dis lved du ing th embedding and th section incubated i senti lly a one-compartment sy tem through which th component of th incubation medium and primary and final rea tion prod ts can diffus Th diffusion rates a e undoubtedly con ider bly lower than in wat The wat r occupies about 30 - 40 per cent of the wat r

soaked resin Thus the diffusion rates of small water soluble molecules and ions would be expected to be lowered about 15 - 30 times (Meares 1968) The diffusion of larger molecules and especially crystallization nuclei would of course be much more hampered (White et al 1961)

The method lso has its weaknesses and limitations Some of these will be d tailed and discussed below

The difficulties ar most obvious with r gard to the method for electron microscopy The reason for this is p bably that it is difficult to standardize sufficiently the washing after the incubation Th washing fluid undoubtedly dissolves a minute amount of the precipitate and this of course is especially noticeable in the areas of the section which are least stained Thi is the reason why washing time is reduced compared to the original method This difficulty becomes much more noticeable when sections around 0 1 µm or thinner are u ed In order to obtain highly reprod cible results with regard to the depth of staining the section thickness should not b less than about 0 2 µm

As the section thickness is thus rather high it is essential to use a high acceleration voltage in the electron microscope In most cases 100 kV has been used but th resolution is noticeably improved by the use of 200 kV

Th lack of r producibility is limited to the above mentioned variability of depth of taining and sometimes lack of staining in the thinnest sections in areas where thicker sections show staining

FALSE LOCALIZATION

When the Hans on method is used mainly to examin the cellular distribution of enzyme th question f specificity i the most important one When the aim of th work i t tudy the intra- llul r distribution and the question f specificity is satisfa torily answered the pos ibility of false localization of th stain comes t the fo eground Th general question f fal e localiz tion in histochemistry has been much discussed The terminology and general concepts used by Holt t al (1958) H lt (1959) Pear (1968) and especially van Duijn (1974) a used in th f llowing discu sion

Ridderstrål (1976) discussed the question f fal localization f st in to ell membrane in th frog kidney If memb anes ontain p eformed crystallization nuclei it seems natu al t expect that these hould be equally distributed all ov r the membran Howev r in one cell type the tain i localized at th pical-late al cell membrane and in another ll type only the baso-l teral membrane is stained Thi rgues gainst the st ining being due t f ls localization This line of reasoning is of cours equally applicable with egard t the r sult gained with avian and mammalian kidney

Howev another type f evidenc appear possibl Acc rding to Han son (1968) the taining is due to p ecipitation of a Co-compound as a result f local alkali ation at the enzyme it s When th incubation fluid i l ft in cont ct with the air for som tim th los of carbon dioxid lead to a general alkalization of the perfi i l lay r f th fluid Thi becomes vi ibl by th formation of a rf ce film of precipitate It ppears reasonabl to assume that the stain and the surfac film r due to persaturation with the same Co-compound It should then be pos ibl to demonstrat th di tribution f pr formed cry talliza-

tion nuclei in sections by incubating them on such supersaturated solutions

Secti ns w re floated on the surface of an acetazolamide solution for 45 minutes and then transferred to incubation medium containing acetazolamide Sections were picked up at various times and f rther processed Up to about 15 minutes such sections showed very faint diffus staining At 20 minutes and especially at 30 minutes (when a surface film w s clearly visible) a pronounced staining is obs rved In the rat kidney the stain is composed of a weak diffuse stain about equally strong all through the cyt plasm and a strong nuclear stain which apparently equally affects all nuclei in th s ctions In the proximal tubule the brush border shows clear but much weaker staining In the pigeon the picture is similar Of the staining appearing after prolonged incubation of inhibited sections only the staining of the brush border in the rat is at all simil r to the staining occurring afte short incubation times without inhibitor

Clearly whenever a cell contains hi tochemically detectable carbonic anhydrase the nucleus may become stained whether the nucl us itself contains enzyme or not This must especially be so in the cas of the original m thod After embedding in resin and with thin sections the possibility of long range diffusion should be much less

Wistrand & Kinn (1977) and Wist and (1979) have shown that th r is a separate membrane-bound isoenzym in the kidney This of course is exc llent evidence that the membrane associated stain is due at least partly to enzymatic activity at this very site

ULTRASTRUCTURE OF THE PRECIPITATE AND RESOLUTION OF THE METHOD

With inc easing incubation time th precipitate rapidly becom e v ry den e and then no finer tructure can usually be observed in it with th le tron mi o ope In the early tag of incubation the memb an staining i howev r n t only very thin but is also distinctly pat hy Sometimes ing-lik structur usually with a diamete of about 400 - 500 A i observed Such tructure have been seen in th membranes f the thick tubul of th n phron in th hrew So ex minutus in th membran overing microvilli in th colon f the rabbit (Figs 5 6) and in the mammary gland of th at Occa i nally th taining i thin enough to llow the fin tructure of such rings t be observed They are omposed f a numb r of small dots (Fig 6)

It d es not appear pos ible to decid if thes ing-like structure a e tifact f the method or pos ibly the result of preformed rystallization sites or if they giv true pictu of the arrangement of the enzyme molecul s In any ca they tt t to the high r solution po sibl with th method In Fig 6 the resolution i about 25 A It would appear that the method may giv an unusually harp localization The reason for this is probably the thinness of the sections incubated and the redu ed mobility in the resin of the involved ions and cry tal nucl i

Despit the harpness of localization it i usually impossible t decide whethe a thin p ecipitat lie on th inside or th out ide of a c ll membrane Occasionally when there is tain on both sides of a c ll membrane this can be bserved with c rtainty (Fig 4)

P edom nance of membran staining

One of the main results of the investigation of kidneys with the present method is that the staining is to a very considerable extent localiz d to or closely associated with cell membranes Only in a few c ll types do s cytopl smi staining reach an intensity resembling that normally occurring at most stained membrane This circumstance may appear to be at variance with the circumstanc that the major part of renal carbonic anhydrase is cytoplasmic

This problem was discussed by Ridderstråle (1976) who gave several possible explanations One is that the method may be too in ensitive to show any staining by a large amount of cytoplasmic enzyme di trib ted in a large volume whereas a small amount of membrane-associ ted activity in a small volume could reach a concentration high enough to give strong staining Other possibl explanation considered w re differential inactivation of membrane-associated and cytopl smic enzyme during fixation or embedding For su h explanations there appeared at that time to be little f undation

With th more extensive knowledge of the r sults of the method now avail bl and espe ially after the discovery of a membrane-bound isoenzyme f rbonic anhydrase in the kidney (Wistrand et al. 1977 and Wist and 1979) it appears possible to discuss the problem mor thoroughly

A very rough omparison f the enzyme concentrations in membran s with tho e f the cytoplasm may be made in mammalian kid eys W lling t l (1975) give the surface of membranes in th rabbit p oximal t bul s as 8 μm^2 / μm^3 The proximal tubule ll undoubt dly has much higher surface to volume ratio than

other renal cells thus this rati may perhaps be taken as $5\ \mu m^2 / \mu m^3$ f r the entire kidney As using th membran thi k ness t be 30 – 100 A the atio membran volume / cell volume would be 1/20 – 1/70 The enzyme activity in membrane and mi rosomes is about 5 per cent f tot l a tivity according t Wi t and (1979) Thus th enzyme activity would probably be only slightly higher in membranes than in cytoplasm.

It then appear reasonabl to look at other pos ibiliti of expl ining the usually much high density of stain associated with membrane than with cytoplasmi reas

One f ctor that may ontribut is diff rential d truction of a tivity d ing fixation It ppears quit po sibl that enzyme molecules embedded in lipid could be l s affected than the cyt plasmic enzyme

The passage of the fixed tis u through increasing concentration f monome and perhaps espe ially the hort heating in the polym rizing mixture ould lso res lt in differential inactivation In this ca also it ppears reasonabl that the cytoplasmic enzyme could be more affected than th membrane-bound

That inactivation of enzyme probably occurs d ring embedding i lear from a comparison of th re ult of Lönn rholm & Ridd strål (1974) and Ridderstrål (1976) At equal times of incubation th original Han son method gav much l rg r t tal amount of precipitat than th method with r in embeddi g It is improbabl that this consid rabl diff rence an b explained by th ther fact rs whi h e diff rent b tween the two proced es

Provided the enzyme activity i higher in th membranes ther i y t an th ircumstanc which may ontribute substanti lly t gi an impre sion f predominance of memb an taining

The stained memb an ften show di tin t tendency t g t thick th longe th inc bation time and in some i stances fa rly broad are in th vi i ity of tained membran s g t

stained This is seen very clearly in Figs 3 & 4 of Ridderstrâle (1976) This phenomenon may of course simply be the result of diffusion of primary and final reaction products from the site of high enzyme activity in the membrane It appears however that it may ft n b the result of the additive ff ct of such diffusion and the products of cytoplasmic activity though the latter is in itself insufficient to result in staining Thus cytoplasmic enzyme activity may contribute to the impr ssion of predominant membrane st ining This eff ct would b expected to increase in importance the thicker the section

Clearly th r are several mechanisms which may give the result that cytoplasmic staining is considerably weaker than what corr s ponds to the lev ls of cytoplasmi enzym If this is true it represents a weakne f the method At the same time this weakness may be a necessary p erequisite for the sharp and clear demonstration of membrane-bound enzyme which is the type of enzyme most lik ly t show an interesting intr llular distribution

In any case this admittedly hypothetical discussion may serve to how that the m thod with incubation after embedding may for some purposes be less suitable than the original Hansson method nd that sometimes a combination of methods is needed

Survey of carboni anhydrase di tribution in the kidneys of frog swift and rat

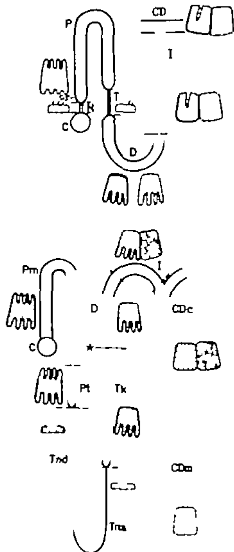
Th distribution of carbonic anhyd se acc rding to the esult of Ridd rstrål (1976 1980) and Lönne holm & Ridder tråle (1980) can best be ondensed in schematic pi tures shown in Fig 1
For t rmin logy see pages 17 - 18

Fig 1 Short remarks and explanations of the symbols

Frog Occasionally the brush border in the proximal tubule shows activity The distal tubule contains a segment with intense membrane staining especially its luminal membranes The rest of the distal tubule is unstained and the exact location of the stained segment is not known

Bird. The brush border of a minor part of the proximal tubules shows weak activity In the thick tubule the lateral cell membranes are stained and in cortical nephrons also the apical cell membrane The distal tubule contains the most heavily stained cells in the avian nephron with intense activity in the luminal membranes Cytoplasmic activity is also found here In the rest of the nephron the staining of the baso-lateral membranes predominates

Rat The dark cells in the initial collecting tubule and cortical duct show the strongest cytoplasmic staining but also the main segment shows considerable cytoplasmic activity The trongest membrane-bound activity is found in the thick tubule and main segment.



FROG
CD
P
I
T
C
D
AVIAN CORTICAL NEPHRON
CDc
P
Tk
I
C
D
Pm
I
D
CDc
C
Pt
Tk
Tnd
CDm
Tna
RAT
D
I
C
P
Tk
Tn
CDm
AVIAN MEDULLARY NEPHRON

Capsule C	*Initial collecting tubule I*
Neck N	*Collecting duct CD*
Proximal tubule P	*cortical duct CDc*
main segment Pm	*medullary duct CDm.*
terminal segment Pt	
Thin tubule Tn	*—— no membrane staining*
descending segment Tnd.	*—— weak membrane staining*
ascending segment Tna	*—— strong membrane staining*
Thick tubule Tk	*: weak cytoplasmic staining*
*Macula densa **	*: :: strong cytoplasmic staining*
Distal tubule D	

NOTE ON THE TERMINOLOGY OF THE NEPHRON AND THE DUCT SYSTEM

In th pap s forming the basis of this the is everal diff ent systems of designating the parts f the nephron hav been used In Ridd ratràl (1980) and in the present paper the t rminology devi tes in some respects from what may appear most common in this rather onfusing fi ld It ppear appropriate at this point to explain very shortly th point f view which ar at the ba i of thi nomenclatu

The overwhelming maj ity f renal research wo k deal with the mammalian kidney Thus any sy tem of terminol gy hould b conveniently pplicable to that nephron and hould b based on morphological featu es as these are most unive sally ascertainabl

The nephron is supposed t end at th point where it j ins another nephron o collecting duct

The major divisions of th nephron are designated tubul the divisions of tubul s re d ignated segment The divisions of th ollecting system re d ignated duct

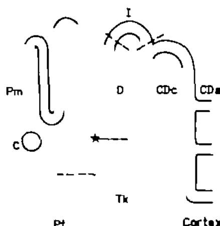
Whenev r possible any term should b composed of two words only or if necessary for clarity at most three words are used

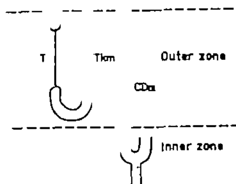
The designations of the segments are chosen so that the segment can be unequivocally described without reference to the tubule it is a part of

When applied to the mammalian kidney the system works out as shown in Fig 2

Fig 2

Capsule C
(Neck N)
Proximal tubule P
  main segment Pm
  terminal segment Pt
Thin tubule Tn
  descending segment Tnd
  ascending segment Tna
Thick tubule Tk
  medullary segment Tkm
  cortical segment Tkc
  macula densa
  (intercalated segment Tki)
Distal tubule D
Initial collecting tubule I
Collecting duct CD
  ascending duct CDa
  cortical duct CDc
  medullary duct CDm





It p haps needs emphasizing that the t rminal s gment is not equivalent to the str ight part of the proximal tubul The boundary b tw en the convolutions and the traight part does usually n t coincide with th chang in cytology which i the boundary between main s gment and t rminal segment

In mo t ases ther i no int rcalated segment When ther is on it i perhaps b t in luded in the thick tubule on the basis of it cyt logy (Kaissling t 1 1977)

The neck is illiated part of the n phron occurring in lower vertab ates wher the thin tubul is als illiated

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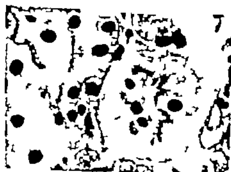


Fig 3 Section from cortex of rat kidney Inhibited by acetazolamide incubated 30 min Strong unspecific staining of nuclei weaker of brush border 640 X



Fig 4 Erythrocyte of Sorex minutus showing carbonic anhydrase activity on both sides of the cell membrane 18 600 X



Fig 5 Microvilli from surface epithelium of rabbit colon showing ring-like staining pattern. 34 000 X.

Fig 6 Similar to Fig 5 124 000 X

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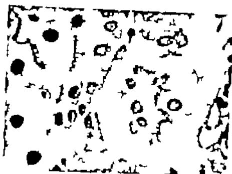


Fig 3 Section from cortex of rat kidney Inhibited by acetazolamide incubated 30 min. Strong unspecific staining of nuclei weaker of brush border 640 X



Fig 4 Erythrocyte of Sorex minutus showing carbonic anhydrase activity on both sides of the cell membrane 18 600 X



Fig 5 Microvilli from surface epithelium of rabbit colon showing ring-like staining pattern. 34 000 X

Fig 6 Similar to Fig 5 124 000 X

Indirect support according to Dreyfus (1976) for the view that the symptoms of thiamine deficiency are not the result of an impaired coenzyme function of Th DP is that the deficiency symptoms are much easier precipitated if the thiamine deficiency is combined with a large carbohydrate load The effect of the carbohydrate load was considered to be due to stimulation of the enzyme systems where Th DP is a coenzyme at the expense of another thiamine - requiring mechanism that is responsible for the deficiency symptoms

A possible function of thiamine in neuromuscular transmission was investigated in the present work by studying

1 the formation of the methylthiamine-like substance (MTLS) from choline and thiamine

2 the formation of the MTLS after nerve stimulation

3 the release of radioactive thiamine from the stimulated phrenic nerve-diaphragm preparation

4 the binding and effect of thiamine on nicotinic acetylcholine receptors and

5 the effect of thiamine on neuromuscular transmission in rat masseter muscle

COMMENTS ON METHODS

The different methods have been described in detail in the individual papers and therefore the methodological principles are only shortly summarized here

In paper I and II the methods for the extraction of the MTLS are given The MTLS was extracted from homogenized cat ventral roots using a counter ion dissolved in an organic solvent The homogenization was carried out in an organic solvent which has two advantages immediate denaturation of the proteins and a partial solubilization of the lipids These facts may explain why the MTLS was not found in earlier studies where choline metabolites have been extracted first after acid precipitation Accordingly when the ventral roots in the present study were homogenized in trichloroacetic acid no MTLS was recovered from the supernatant after centrifugation The occurrence of MTLS in the organic homogenization medium was considered improbable since only trace amounts of MTLS were found if radioactive line was added to nonradioactive ventral roots before the homogenization

In paper V the binding of thiamine to nicotinic acetylcholine receptors was studied both by using an isolated receptor protein from the electric organ of Torpedo marmorata and by using end plate receptors from frog sartorius muscle The isolated nicotinic acetylcholine receptor has certain characteristics in common with nicotinic receptors in the frog muscle end plate it binds nicotinic antagonists in low concentrations However the binding of acetylcholine to the isolated nicotinic acetylcholine receptor requires a high acetylcholine concentration (10^{6} M) whereas the

acetylcholine concentration that has an effect on the frog end-plate is much lower (10^{-9} M) Since differences in binding characteristics between the two types of receptors exist it is important to complete results achieved with the isolated receptor with results from frog muscle end plate receptors If the binding of a substance to nicotinic receptors is studied by using both techniques and the results are the same then the probability is increased that the binding of this substance to nicotinic acetylcholine receptors or to structures closely related to their function (ionophores) is studied

In paper VI the effect of thiamine on post-tetanic potentiation (PTP) was studied using the masseter muscle of the rat The masseteric nerve was stimulated with supramaximal impulses the voltage of the stimulation impulses being 150 % of that which yielded maximal muscle twitches Therefore the observed changes in muscle tension of the muscle contractions do not depend on differences in the recruitment of muscle fibres In some experiments field stimulation of the masseter muscle was made No difference in results between stimulation of the masseteric nerve and field stimulation was noted The pre tetanic and post-tetanic stimulation frequency was 0 2 Hz and the impulse duration 0 2 ms When the low frequency stimulation is interrupted by a short tetanus a transitory increase in the peak tension of the isometric muscle twitch response (PTP) is seen in fast mammalian muscle PTP in fast mammalian muscle has been described to be due to an increased contractility of the muscle fibres (Standaert 1964)

RESULTS AND DISCUSSION

Chromatographical characterization of the MTLS

When the radioactive ^{3}H-choline was injected into the ventral horn nuclei in the spinal cord of the cat radioactivity appeared in the ventral roots after less than 30 min (paper III) Part of the radioactivity was extracted by homogenizing the roots in allylcyanide containing sodium-tetraphenyloboron (counter ion extraction) Compounds which contain a quaternary ammonium ion as the single charge are thereby bound to the sodium-tetraphenyloboron and extracted into the organic phase (Fonnum 1969) By *adding a strong acid to the organic phase (e g HCl) the* quaternary ammonium compounds are regained into the inorganic phase which thereafter can be freeze-dried

When the freeze dried extracts from the radioactive ventral roots were dissolved in ethanol and submitted to paper chromatography then three peaks of radioactivity could be found Two peaks had as expected the chromatographical characteristics of acetylcholine and choline but the third one could not be identified as any known choline metabolite Thiamine was found to be the only substance with chromatographical properties similar to those of the unknown substance Since the methyl groups of the quaternary ammonium ion of choline were labelled the possibility was considered that the unknown compound was identical with methylthiamine formed by methylation

Methylthiamine was synthesized as described in paper III and chromatographed together with the unknown compound in

several chromatographical systems

In all chromatographical systems tried the unknown compound behaved as methylthiamine Radioactive thiamine was injected into the ventral horn nuclei Also in this case a radioactive peak could be found in the chromatograms of ventral root extracts Thus a compound (methylthiamine-like substance (MTLS) with the same chromatographical characteristics as methylthiamine may have been formed from both choline and thiamine The evidence for identity between the MTLS and methylthiamine is 1 chromatographical identity between the MTLS and methylthiamine in several chromatographical systems and 2 the MTLS is formed both from choline and thiamine Such evidence for identit between two compounds is only indirect and therefore the substance is called MTLS

MTLS and choline metabolism

The MTLS was formed in the ventral roots after the injection of radioactive choline It was considered to be of interest to study whether the MTLS was formed from choline itself or from any of its metabolites (paper III) Choline is metabolized in three directions 1 to acetylcholine 2 to phospholipids and 3 to betaine

Radioactive betain did not serve as a precursor for the MTLS when injected into the ventral horn nuclei Therefore it was considered improbable that the MTLS was formed after demethylation of betain or from any of its metabolites To study a possible relationship between the formation of the MTLS and phospholipids the time course for formation of the MTLS and phospholipids was compared The formation of

the MTLS was maximal after two hours Thus the formation of the MTLS does not seem to be dependent on the formation of phospholipids When the amounts of acetylcholine and MTLS in ventral and dorsal roots were compared the amounts of both acetylcholine and MTLS were higher in ventral roots than in the dorsal roots When the time course of the formation of acetylcholine and MTLS in ventral roots was studied it was found that both compounds reached their maximal level in about 30 min Thus it seems as if the formation of acetylcholine and of MTLS are related The immediate precursor for the MTLS can according to these studies be either choline or acetylcholine The biological importance however of the formation of the MTLS was unclear The idea was put forward that the formation of methylthiamine could reflect the activation of nicotinic receptors

The formation of the MTLS following administration of ^{3}H choline did not occur in liver kidney or spleen and may therefore be specific for nerve tissue and especially motor nerves Since it is not formed in all tissues it is improbable that the formation of the MTLS only represents a common break-down product of choline or thiamine

Formation of the MTLS in the stimulated rat hemidiaphragm
To investigate whether the formation of the MTLS could be influenced by changes in nerve activity (as was the formation of compound X in v Muralt s study) the formation of the MTLS in the stimulated and nonstimulated phrenic nerve-dia phragm preparation was studied It was reported in paper IV that the amounts of MTLS formed were found to be higher in stimulated that in unstimulated rat diaphragms The formation of the MTLS was inhibited by d tubocurarine (12 mg/kg) The MTLS could only be extracted from the nerve

terminal region of the diaphragm muscle (can easily been seen as a band along the muscle) Thus the level of MTLS in the innervated part of the rat diaphragms increased as a result of nerve stimulation as did compound X in v Muralt s study (1958) In addition the formation of MTLS was inhibited by curare suggesting that the formation of the MTLS is linked to the activation of curare-sensitive receptors

Release of ^{35}S-thiamine from the stimulated rat diaphragm When the phrenic nerve of the hemidiaphragms was stimulated a release of radioactive thiamine into the bath was found in the presence of d-tubocurarine (paper IV) Since the muscle contractions were blocked by d tubocurarine the thiamine in the bath after nerve stimulation probably originated from the nerve fibres Thiamine is mainly stored as phosphates Therefore a dephosphorylation of thiamine or an increase in extractable non phosphorylated thiamine seems to occur during nerve stimulation In electrically stimulated frog sciatic nerves v Muralt (1947) found an increase in thiamine extracted by Ringer solutions results which showed that the amount of free (=extractable) thiamine had increased as a result of the stimulation A dephosphorylation of thiamine phosphates was noted by Itokawa and Cooper (1970) who found an increase in non phosphorylated thiamine in the perfusion fluid from rat sciatic nerve or rat spinal cord when neuroactive agents such as acetylcholine tetrodotoxin ouabain or LSD were added to the perfusion fluid Thus electrical stimulation of or addition of certain neuroactive agents to nervous tissue might induce a shift from bound to free thiamine and /or/ a dephosphorylation of the thiamine phosphates

Binding of thiamine to nicotinic acetylcholine receptors
Since the increase in the MTLS in the phrenic nerve-diaphragm preparation was blocked by d-tubocurarine it was considered to be of interest to study a possible binding to and effect of thiamine on nicotinic acetylcholine receptors Nicotinic acetylcholine receptors can be isolated and purified from electric organs of fish (Eldefrawi and Eldefrawi 1973 Heilbronn et al 1974 Klett et al 1973 Meunier et al 1974) Such material was considered to be suitable for binding studies In paper V it was shown that thiamine binds reversibly to the isolated nicotinic acetylcholine receptor protein (K_D=10^{5} M) The binding of thiamine was inhibited by the venom toxin from *Naja naja siamensis* which is a specific antagonist for postsynaptic nicotinic receptors (Chang and Lee 1966) The binding of thiamine to the isolated nicotinic receptor was not inhibited by acetylcholine

A possible effect of thiamine on post-synaptic nicotinic acetylcholine receptors was investigated by studying the miniature end plate potentials of the frog muscle end plate (paper V) The miniature end plate potentials are the result of nicotinic acetylcholine receptor activation due to release of acetylcholine When thiamine (10^{5} M) was applied to the end plate preparation the amplitude of the miniature end plate potentials decreased reversibly and significantly No effect on the resting membrane potential was noted The decrease in the miniature end plate potentials induced by thiamine indicated that thiamine blocks the action of acetylcholine A decrease in the miniature end plate potentials by thiamine can be induced by a block of the sodium channels in the end plate that give rise to the miniature end plate potentials registered Since the binding of acetylcholine

was not inhibited by thiamine in the isolated receptor from Torpedo marmorata these results could indicate that thiamine binds to the sodium channels and not to the acetylcholine binding site

Pyrithiamine and neuromuscular transmission

In paper VI the effect of thiamine on neurotransmission at higher stimulation frequencies (> 1Hz) was studied in rat masseter muscle It is a typical fast muscle since it responds with individual twitches up to frequencies of 30 Hz Since the masseter muscle is fast the tension of the individual muscle twitches will increase at high stimulation frequencies (stair case effect) and a frequency-dependent increase in contractility (PTP) will develop after moderate tetanic stimulations

The effect of thiamine on the development of PTP in the untreated masseter muscle was investigated by administering thiamine intravenously No effect was noted on the PTP or on the ordinary single twitches When the effect of pyrithiamine and thiaminase-containing preparations (fern extract) on the PTP was studied then the muscle twitches at stimulation frequencies of > 1 Hz were decreased and PTP was abolished Intravenous injections of thiamine alone there after restored the PTP and the muscle twitches at high frequency stimulation Oxythiamine had no effect on the muscle twitches induced by high frequency stimulation or on the PTP No effect on directly excited muscle twitches was seen after pyrithiamine administration Therefore the effects of pyrithianine and thiamine are on the nerve and not on the muscle

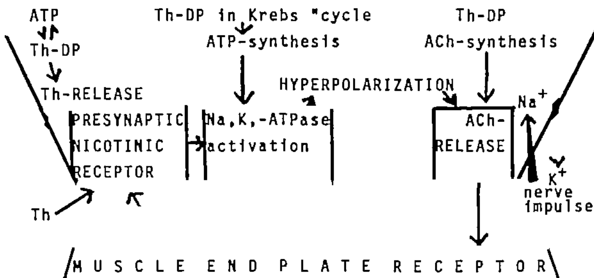
Pyrithiamine administration caused a concommittant decrease in the PTP and the muscle twitches at stimulation frequencies of > 1 Hz The effect of pyrithiamine on neurotransmission at high stimulation frequencies is due to an impaired neuromuscular transmission that is necessary for the development of the frequency-dependent increase in contractility (staircase and PTP) since the directly-stimulated muscle twitches at high frequency stimulation were unaffected

The impairement of neurotransmission induced by pyrithiamine that was reversed by thiamine could be due to a decreased excitability of the presynaptic nerve fibers or /and/ of the end-plate membrane Metabolic effects such as a decreased synthesis of acetylcholine or ATP or impairement of a specific function of thiamine in the nerve membrane could be the cause for the decrease in excitability

Possible mechanisms of action of thiamine

A possible function of thiamine in neurotranswission is its role as a coenzyme for pyruvate dehydrogenase an enzyme which regenerates acetyl groups and which therefore is of importance for acetylcholine synthesis and for the regeneration of ATP in the citric acid cycle However experimental evidence does not support the view that such a mechanism of action of thiamine is of importance for the symptoms in thiamine-deficient animals In thiamine deficiency the acetylcholine levels are described to be either elevated or decreased (Gubler 1976) and the ATP-levels normal or elevated (Holowick et al 1968 McCandless and Schenker 1968 Inoue et al 1970) In the present study d-tubocura rine which blocks the effect of acetylcholine, induced a similar decrease in the PTP and reversal of the stair case

effect as did pyrithiamine This result however could be consistent with the view that pyrithiamine inhibits a function of acetylcholine in the masseter muscle preparation that is necessary for the development of PTP and maintenance of muscle twitches at stimulations above 1 Hz If thiamine deficiency or pyrithiamine administration do not change the levels of acetylcholine or the energy supply (ATP production) another possibility could be that thiamine is necessary for the change in nervous excitability induced by acetylcholine Pyrithiamine has been shown to inhibit the post tetanic hyperpolarization (Cooper and Pincus 1967) without affecting the Na K_rATPase results which suggest that thiamine is of importance for maintenance of the membrane potential at high stimulation frequencies Acetylcholine has been shown to increase the hyperpolarization in C-fibers of rabbit vagus nerve (Armett and Ritchie 1960) This effect of acetylcholine was inhibited by d-tubocurarine and hexamethonium but not by atropin in corresponding doses Thus both acetylcholine and thiamine might be of importance for maintaining the membrane potential at high frequency stimulation The efficiency of neurotransmission is directly dependent on the degree of membrane polarization (Eccles 1964) Therefore it is possible that acetylcholine and pyrithiamine also are able to affect the release of transmitter In accordance with this view are the results where acetylcholine has been shown to be important for maintaining the neurotransmission at higher stimulation frequencies via a presynaptic action (Bowman and Webb 1973 Beranek and Vyskocil 1967 Hubbard et al 1969) Thiamine could as already mentioned be of importance for the synthesis of acetylcholine and /or/ ATP and thus influencing the effects of acetylcholine on the membrane potential If however



(ACh=acetylcholine Th=thiamine and Th-DP= thiamine diphosphate)

Fig 1 Thiamine and neuromuscular transmission
Possible sites of action for thiamine
A schematic picture of the presynaptic nerve terminal and the muscle end plate is shown Possible mechanisms of action for thiamine in maintaining the neuromuscular transmission are illustrated The different mechanisms of action for thiamine are described in the text under results and discussion

pyrithiamine blocks a function of thiamine which is unrelated to the thiamine-requiring enzymes then it is possible that thiamine is of importance for the excitability of presynaptic structures (nicotinic receptors) activated by acetylcholine that are of importance for maintaining neurotransmission at high stimulation frequencies

In paper III the hypothesis was put forward that the formation of the MTLS may reflect the activation of nicotinic receptors Evidence for such a hypothesis should include 1 decreased formation of the MTLS in the presence of d tubocurarine 2 a binding of thiamine to nicotinic receptors and 3 an effect of thiamine on the function of nicotinic receptors A decreased formation of the MTLS after administration of d tubocurarine was shown in paper IV and a specific binding to nicotinic receptors in paper V However addition of thiamine to nicotinic receptors of the frog end plate decreased the miniature end plate potentials Thiamine was also specifically bound to an isolated nicotinic receptor protein from <u>Torpedo marmorata</u> a binding which was inhibited by a venom toxin with specific affinity to nicotinic receptors but not by acetylcholine Thus these results suggest that thiamine was bound to the sodium channels necessary for the miniature end plate potentials The specific binding of thiamine to the acetylcholine-activated sodium channels might imply that thiamine is important for the activation of these channles in lower concentrations Unfortunately this possibility can not be supported or disclosed by the experiments in paper V since an experimental thiamine deficiency was not induced

Any direct evidence showing that formation of the MTLS reflects activation of nicotinic receptors was not obtained in the present study The experiments performed on rat masseter muscle shows that the effects of pyrithiamine and d-tubo-curarine on the stair-case effect and the PTP are similar and are possible to explain if it is assumed that thiamine is of importance for a presynaptic action of acetylcholine necessary for the membrane potential

In paper IV an increase in nonphosphorylated thiamine was found in the stimulated phrenic nerve diaphragm preparation Similar results were described by v Muralt (1947) from stimulated frog nerve Thus electrical stimulation of nerve tissue seem to increase the amount of nonphosphorylated thiamine suggesting an increased turn over of thiamine at electrical stimulation These results can be in accordance with the view that thiamine compounds are of importance for miantaining the membrane potential presumably by influencing the ion transport through the membrane since electrical stimulation will activate the mechanisms that are necessary for maintaining the membrane potential

An increase in an unidentified thiamine compound (compound X) after nerve stimulation has been described by v Muralt (1958) An increase in a thiamine metabolite (MTLS) after nerve stimulation is described also in this study These results also indicate that there is an increase in the turnover of thiamine at nerve stimulation

In the present work it was found that the increase in the MTLS seen after nerve stimulation was inhibited by d tubo curarine A frequency dependent decrease in the excitability

of the nerve terminal fibres is seen after d tubocurarine administration (see paper VI for references) Thus these results are in accordance with the view that there is a turn-over of thiamine compounds in nerve tissue that is related to membrane excitability

The present results support the hypothesis that thiamine is of importance for membrane excitability especially at neuromuscular synapses and that there is a turn-over of thiamine compounds In the present study it has been shown that the neuromuscular transmission is impaired at an early stage by the thiamine antagonist pyrithiamine If pyrithiamine is antagonizing a function of thiamine necessary for membrane excitability then the neuromuscular transmission apparently is the most vulnerable localization for a dysfunction of the peripheral motor system due to pyrithiamine administration

The importance of a synaptic dysfunction also in thiamine deficiency whatever the biochemial mechanism behind the dysfunction may be is supported by the morphological studies carried out on brains from thiamine deficient rats A hypertrophy of the synaptic boutons and a decrease in the number of synaptic vesicles were shown in thiamine-deficient rats (Tellez and Terry 1968 Pena and Felter 1973) In addition the importance of synaptic failure in thiamine deficiency may also be supported by the fact that the symptoms in the early stages of thiamine deficiency can be reversed within a few hours (Dreyfus 1976) Thiamine reaches the axon membranes via axoplasmic transport (Tanaka et al 1976) and it is improbable that thiamine which is a quaternary amine could reach the axon membrane by passive diffusion Since

the deficiency symptoms are reversed within a few hours and the axoplasmic transport of thiamine to the entire axons probably will take place in a much longer time, the most probable site of membrane dysfunction in the early stages of thiamine deficiency is in the terminal boutons which are unprotected by myelin and thus accessible to the administration of thiamine

CONCLUSIONS

1 The formation of a methylthiamine-like substance (MTLS) in nerve tissue is described

2 The formation of the MTLS reached its maximum at the same time as the formation of acetylcholine but significantly earlier than the formation of phospholipids No MTLS was formed from betain

3 The formation of the MTLS increased with nerve stimulation and was inhibited by d-tubocurarine The role of the formatior of the MTLS remains unknown However it is an interesting possibility that the formation of the MTLS is an indicator of a specific role of thiamine for membrane excitability

4 Thiamine is released from the curarized phrenic nerve-diaphragm when the phrenic nerve is stimulated

5 Thiamine binds to isolated nicotinic acetylcholine receptors from Torpedo marmorata and to nicotinic acetylcholine receptors of the frog end plate

6 Pyrithiamine and fern extract thiamine antagonists impaires neurotransmission in rat masseter muscle via a frequency dependent mechanism The effect of pyrithiamine and fern extract is reversed by thiamine Oxythiamine has no effect on the neurotransmission in rat masseter muscle

7 The present results are consistent with the view that thiamine is important for the excitability of the nerve terminals by an effect on the active ion transport that is necessary for regeneration and maintenance of the membrane potential

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